

**TITLE:** **POTATO TRANSCRIPTION FACTORS, METHODS  
OF USE THEREOF, AND A METHOD FOR  
ENHANCING TUBER DEVELOPMENT**

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**POTATO TRANSCRIPTION FACTORS, METHODS OF USE THEREOF,  
AND A METHOD FOR ENHANCING TUBER DEVELOPMENT**

[0001] The present invention claims benefit of U.S. Provisional  
Application Serial No. 60/397,423, filed July 19, 2002, which is hereby  
5 incorporated by reference in its entirety.

[0002] The subject matter of this application was made with support from  
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**FIELD OF THE INVENTION**

[0003] The present invention relates to isolated BEL transcription factors  
from *Solanum tuberosum*, a method of enhancing tuber development in plants,  
and methods of regulating flowering and growth in plants.

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**BACKGROUND OF THE INVENTION**

[0004] The primary developmental events of plants originate from the  
shoot apical meristem (SAM) (Clark, "Organ Formation at the Vegetative Shoot  
Meristem," Plant Cell 9:1067-1076 (1997); Kerstetter et al., "Shoot Meristem  
Formation in Vegetative Development," Plant Cell 9:1001-1010 (1997)). The  
20 shoot apical meristem (SAM) is responsible for the formation of vegetative organs  
such as leaves, and may undergo a phase change to form the inflorescence or  
floral meristem. Many of these events are controlled at the molecular level by  
transcription factors. Transcription factors (TFs) are proteins that act as  
developmental switches by binding to the DNA (or to other proteins that bind to  
25 the DNA) of specific target genes to modulate their expression. An important  
family of TFs involved in regulating the developmental events in apical meristems  
is the *knox* (knotted-like homeobox) gene family (Reiser et al., "Knots in the  
Family Tree: Evolutionary Relationships and Functions of Knox Homeobox  
Genes," Plant Mol Biol 42:151-166 (2000)). *Knox* genes have been isolated from  
30 several plant species (reviewed in Reiser et al., "Knots in the Family Tree:

Evolutionary Relationships and Functions of *knox* Homeobox Genes," Plant Mol. Biol. 42:151-166 (2000)) and can be divided into two classes based on expression patterns and sequence similarity (Kerstetter et al., "Sequence Analysis and Expression Patterns Divide the Maize *knotted1*-like Homeobox Genes into Two

5 Classes," Plant Cell 6:1888-1887 (1994)). Class I *knox* genes have high similarity to the *kn1* homeodomain and generally have a meristem-specific mRNA expression pattern. Class II *knox* genes usually have a more widespread expression pattern.

[0005] *Knox* genes belong to the group of TFs known as the TALE

10 superclass (Bürglin, "Analysis of TALE Superclass Homeobox Genes (MEIS, PBC, KNOX, Iroquois, TGIF) Reveals a Novel Domain Conserved Between Plants and Animals," Nucleic Acids Res 25:4173-4180 (1997)). These TFs are distinguished by a very high level of sequence conservation in the DNA-binding region, designated the homeodomain, and consisting of three  $\alpha$ -helices similar to

15 the bacterial helix-loop-helix motif (Kerstetter et al., "Sequence Analysis and Expression Patterns Divide the Maize *knotted1*-like Homeobox Genes into Two Classes," Plant Cell 6:1877-1887 (1994)). The third helix, the recognition helix, is involved in DNA-binding (Mann et al., "Extra Specificity From *extradenticle*: the Partnership Between HOX and PBX/EXD Homeodomain Proteins," Trends in

20 Genet 12:258-262 (1996)). TALE TFs contain a three amino acid loop extension (TALE), proline-tyrosine-proline, between helices I and II in the homeodomain, that has been implicated in protein interactions (Passner et al., "Structure of DNA-Bound Ultrabithorax-Extradenticle Homeodomain Complex," Nature 397:714-719 (1999)). There are numerous TFs from plants and animals in the TALE superclass

25 and the two main groups in plants are the KNOX and BEL types (Bürglin, "Analysis of TALE Superclass Homeobox Genes (MEIS, PBC, KNOX, Iroquois, TGIF) Reveals a Novel Domain Conserved Between Plants and Animals," Nucleic Acids Res 25:4173-4180 (1997)). Related genes in animal systems play an important role in regulating gene expression.

30 [0006] Expression patterns and functional analysis of mutations support the involvement of *knox* genes in specific developmental processes of the shoot apical meristem. *Kn1* from maize, the first plant homeobox gene to be discovered

- (Vollbrecht et al., "The Developmental Gene *Knotted-1* is a Member of a Maize Homeobox Gene Family," Nature 350:241-243 (1991)), is involved in maintenance of the shoot apical meristem and is implicated in the switch from indeterminate to determinate cell fates (Chan et al., "Homeoboxes in Plant
- 5 Development," Biochim Biophys Acta 1442:1-19 (1998); Kerstetter et al., "Loss-of-Function Mutations in the Maize Homeobox Gene, *knotted1*, are Defective in Shoot Meristem Maintenance," Development 124:3045-3054 (1997); Clark et al., The *CLAVATA* and *SHOOT MERISTEMLESS* Loci Competitively Regulate Meristem Activity in *Arabidopsis*," Development 122:1567-1575 (1996)).
- 10 Transcripts of *kn1* in maize (Jackson et al., "Expression of Maize *KNOTTED1* Related Homeobox Genes in the Shoot Apical Meristem Predicts Patterns of Morphogenesis in the Vegetative Shoot," Development 120:405-413 (1994)), *OSH1* in rice (Sentoku et al., "Regional Expression of the Rice *KN1*-type Homeobox Gene Family During Embryo, Shoot, and Flower Development," Plant
- 15 Cell 11:1651-1663 (1999)), and *NTH15* in tobacco (Tamaoki et al., "Ectopic Expression of a Tobacco Homeobox Gene, *NTH15*, Dramatically Alters Leaf Morphology and Hormone Levels in Transgenic Tobacco," Plant Cell Physiol 38:917-927 (1997)) were localized by in situ hybridization to undifferentiated cells of the corpus and the developing stem, but were not detected in the tunica or
- 20 leaf primordia. Overexpression of *kn1* in *Arabidopsis* (Lincoln et al., "A *knotted1*-like Homeobox Gene in *Arabidopsis* is Expressed in the Vegetative Meristem and Dramatically Alters Leaf Morphology When Overexpressed in Transgenic Plants," Plant Cell 6:1859-1876 (1994)) and in tobacco (Sinha et al., "Overexpression of the Maize Homeobox Gene, *KNOTTED-1*, Causes a Switch
- 25 From Determinate to Indeterminate Cell Fates," Genes Dev 7:787-795 (1993)), resulted in plants with altered leaf morphologies including lobed, wrinkled or curved leaves with shortened petioles and decreased elongation of veins. Plants were reduced in size and showed a loss of apical dominance. In plants with a severe phenotype, ectopic meristems formed near the veins of leaves indicating a
- 30 reversion of cell fate back to the indeterminate state (Sinha et al., "Overexpression of the Maize Homeobox Gene, *KNOTTED-1*, Causes a Switch From Determinate to Indeterminate Cell Fates," Genes Dev 7:787-795 (1993)). Overexpression of *OSH1* or *NTH15* in tobacco resulted in altered morphologies similar to the 35S-



- kn1* phenotype (Sato et al., "Abnormal Cell Divisions in Leaf Primordia Caused by the Expression of the Rice Homeobox Gene OSH1 Lead to Altered Morphology of Leaves in Transgenic Tobacco," Mol Gen Genet 251:13-22 (1996); Tamaoki et al., "Ectopic Expression of a Tobacco Homeobox Gene, *NTH15*, Dramatically Alters Leaf Morphology and Hormone Levels in Transgenic Tobacco," Plant Cell Physiol 38:917-927 (1997)).
- [0007] Alterations in leaf and flower morphology in 35S-*NTH15* or *OSH1* transgenic tobacco were accompanied by changes in hormone levels. Whereas levels of all the hormones measured were changed slightly, both gibberellin and cytokinin levels were dramatically altered (Kusaba et al., "Alteration of Hormone Levels in Transgenic Tobacco Plants Overexpressing the Rice Homeobox Gene *OSH1*," Plant Physiol 116:471-476 (1998); Tamaoki et al., "Ectopic Expression of a Tobacco Homeobox Gene, *NTH15*, Dramatically Alters Leaf Morphology and Hormone Levels in Transgenic Tobacco," Plant Cell Physiol 38:917-927 (1997)).
- RNA blot analysis revealed that the accumulation of GA 20-oxidase1 mRNA was reduced several fold in transgenic plants (Kusaba et al., "Decreased GA<sub>1</sub> Content Caused by the Overexpression of OSH1 is Accompanied by Suppression of GA 20-oxidase Gene Expression," Plant Physiol 117:1179-1184 (1998); Tanaka-Ueguchi et al., "Overexpression of a Tobacco Homeobox Gene, *NTH15*, Decreases the Expression of a Gibberellin Biosynthetic Gene Encoding GA 20-oxidase," Plant J 15:391-400 (1998)). A KNOX protein of tobacco binds to specific elements in regulatory regions of the GA 20-oxidase1 gene of tobacco to repress its activity (Sakamoto et al., KNOX Homeodomain Protein Directly Suppresses the Expression of a Gibberellin Biosynthesis Gene in the Tobacco Shoot Apical Meristem," Genes Dev 15:581-590 (2001)). GA 20-oxidase is a key enzyme in the GA biosynthetic pathway necessary for the production of the physiologically inactive GA<sub>20</sub> precursor of active GA<sub>1</sub> (Hedden et al., "Gibberellin Biosynthesis: Enzymes, Genes and Their Regulation," Annu Rev Plant Physiol Plant Mol Biol 48:431-460 (1997)). GA<sub>1</sub> and other active GA isoforms are important regulators of stem elongation, the orientation of cell division, the inhibition of tuberization, flowering time, and fruit development (Jackson et al., "Control of Tuberisation in Potato by Gibberellins and Phytochrome," B. Physiol

- Plant 98:407-412 (1996); Hedden et al., "Gibberellin Biosynthesis: Enzymes, Genes and Their Regulation," Annu Rev Plant Physiol Plant Mol Biol 48:431-460 (1997); Rebers et al., "Regulation of Gibberellin Biosynthesis Genes During Flower and Early Fruit Development of Tomato," Plant J 17:241-250 (1999)).
- 5    [0008]        Another plant homeobox gene family that is closely related to the *knox* genes is the BEL (BELL) family (Chan et al., "Homeoboxes in Plant Development," Biochim Biophys Acta 1442:1-19 (1998); Bürglin, "Analysis of TALE Superclass Homeobox Genes (MEIS, PBC, KNOX, Iroquois, TGIF) Reveals a Novel Domain Conserved Between Plants and Animals," Nucleic Acids
- 10   Res 25:4173-4180 (1997)). BEL TFs have been implicated in flower and fruit development (Reiser et al., The BELL1 Gene Encodes a Homeodomain Protein Involved in Pattern Formation in the *Arabidopsis* Ovule Primordium," Cell
- 15   83:735-742 (1995); Dong et al., "MDH1: an Apple Homeobox Gene Belonging to the BEL1 Family," Plant Mol Biol 42:623-633 (2000)). Genetic analysis of *BEL1* in *Arabidopsis* showed that expression of this TF regulated the development of ovule integuments and overlaps the expression of *AGAMOUS* (Ray et al., "Arabidopsis Floral Homeotic Gene BELL (*BEL1*) Controls Ovule Development Through Negative Regulation of *AGAMOUS* Gene (*AG*)," Proc Natl Acad Sci USA 91:5761-5765 (1994); Reiser et al., The BELL1 Gene Encodes a
- 20   Homeodomain Protein Involved in Pattern Formation in the *Arabidopsis* Ovule Primordium," Cell 83:735-742 (1995); Western et al., "BELL1 and *AGAMOUS* Genes Promote Ovule Identity in *Arabidopsis thaliana*," Plant J 18:329-336 (1999)). In *COP1* mutants, the photoinduced expression of *ATH1*, another BEL TF of *Arabidopsis*, was elevated, indicating a possible role in the signal
- 25   transduction pathway downstream of *COP1* (Quaedvlieg et al., "The Homeobox Gene *ATH1* of *Arabidopsis* is Depressed in the Photomorphogenic Mutants *cop1* and *det1*," Plant Cell 7:117-129 (1995)).
- [0009]        Plants must maintain a great deal of flexibility during development to respond to environmental and developmental cues. Responses to these signals,
- 30   which include day length, light quality or quantity, temperature, nutrient and hormone levels, are coordinated within the meristem (Kerstetter et al., "Shoot Meristem Formation in Vegetative Development," Plant Cell 9:1001-1010

(1997)). In potato, there is a specialized vegetative meristem called the stolon meristem that develops as a horizontal stem and under inductive conditions will form the potato tuber (Jackson, "Multiple Signaling Pathways Control Tuber Induction in Potato," Plant Physiol. 119:1-8 (1999); Fernie et al., "Molecular and Biochemical Triggers of Potato Tuber Development," Plant Physiol. 127:1459-1465 (2001)). Potato offers an excellent model system for examining how vegetative meristems respond to external and internal factors to control development at the molecular level. In model tuberization systems, synchronous tuber formation occurs under inductive conditions and shoot or stolon formation occurs under noninductive conditions. The cellular and biochemical processes that occur in these model systems have been examined extensively (Vreugdenhil et al., "Initial Anatomical Changes Associated with Tuber Formation on Single-Node Potato (*Solanum tuberosum* L.) Cuttings: A Re-evaluation," Ann. Bot. 84:675-680 (1999); Xu et al., "The Role of Gibberellin, Absciscic Acid, and Sucrose in the Regulation of Potato Tuber Formation *In vitro*," Plant Physiol. 117:575-584 (1998); Hannapel, "Characterization of Early Events of Potato Tuber Development," Physiol. Plant 83:568-573 (1991); Wheeler et al., "Comparison of Axillary Bud Growth and Patatin Accumulation in Potato Leaf Cuttings as Assays for Tuber Induction," Ann. Bot. 62:25-30 (1988)). In addition to being good systems to examine integration of signals at the meristem, understanding the molecular processes controlling tuberization in potato is important. Potato is the fourth largest crop produced in the world, ranking after maize, rice, and wheat, and is a major nutritional source in many countries (Jackson, "Multiple Signaling Pathways Control Tuber Induction in Potato," Plant Physiol. 119:1-8 (1999); Fernie et al., "Molecular and Biochemical Triggers of Potato Tuber Development," Plant Physiol. 127:1459-1465 (2001)); therefore, research focusing on the process of tuber initiation and development is very important.

[0010] Tuber formation in potatoes (*Solanum tuberosum* L.) is a complex developmental process that requires the interaction of environmental, biochemical, and genetic factors. Several important biological processes like carbon partitioning, signal transduction, and meristem determination are involved (Ewing et al., "Tuber Formation in Potato: Induction, Initiation and Growth," Hort. Rev.

- 14:89-198 (1992)). Under conditions of a short-day photoperiod and cool temperature, a transmissible signal is activated that initiates cell division and expansion and a change in the orientation of cell growth in the subapical region of the stolon tip (Ewing et al., "Tuber Formation in Potato: Induction, Initiation and Growth," Hort. Rev. 14:89-198 (1992); Xu et al., "Cell Division and Cell Enlargement During Potato Tuber Formation," J. Expt. Bot. 49:573-582 (1998)). In this signal transduction pathway, perception of the appropriate environmental cues occurs in leaves and is mediated by phytochrome and gibberellins (van den Berg et al., "Morphology and (14C)gibberellin A-12 Metabolism in Wild-Type and Dwarf *Solanum tuberosum* ssp. Andigena Grown Under Long and Short Photoperiods," J. Plant Physiol. 146:467-473 (1995); Jackson et al., "Phytochrome B Mediates the Photoperiodic Control of Tuber Formation in Potato," Plant J. 9:159-166 (1996); Jackson et al., "Control of Tuberisation in Potato by Gibberellins and Phytochrome," B. Physiol Plant 98:407-412 (1996)). Tuber development at the stolon tip is comprised of biochemical and morphological processes. Both are controlled by differential gene expression (Hannapel, "Characterization of Early Events of Potato Tuber Development," Physiol. Plant 83:568-573 (1991); Bachem et al., "Analysis of Gene Expression During Potato Tuber Development," Plant J. 9:745-753 (1996); Macleod et al., "Characterisation of Genes Isolated from a Potato Swelling Stolon cDNA Library," Pot. Res. 42:31-42 (1999)) with most of the work focusing on the biochemical processes, including starch synthesis (Abel et al., "Cloning and Functional Analysis of a cDNA Encoding a Novel 139 kDa Starch Synthase from Potato (*Solanum tuberosum* L.)," Plant J. 10:981-991 (1996); Preiss, "ADPglucose Pyrophosphorylase: Basic Science and Applications in Biotechnology," Biotech. Annu. Rev. 2:259-279 (1996); Geigenberger et al., "Overexpression of Pyrophosphatase Leads to Increased Sucrose Degradation and Starch Synthesis, Increased Activities of Enzymes for Sucrose-Starch Interconversions, and Increased Levels of Nucleotides in Growing Potato Tubers," Planta 205:428-437 (1998)) and storage protein accumulation (Mignery et al., "Isolation and Sequence Analysis of cDNAs for the Major Potato Tuber Protein, Patatin," Nucl. Acid Res. 12:7989-8000 (1984); Hendriks et al., "Patatin and Four serine Protease Inhibitor Genes are Differentially Expressed During Potato Tuber Development," Plant

Mol. Biol. 17:385-394 (1991); Suh et al., "Proteinase-Inhibitor Activity and Wound-Inducible Expression of the 22-kDa Potato-Tuber Proteins," Planta 184:423-430 (1991)).

[0011] Much less is known about the morphological controls of  
5 tuberization, although it is clear that phytohormones play a prominent role (Koda  
et al., "Potato Tuber-Inducing Activities of Jasmonic Acid and Related  
Compounds," Phytochemistry 30:1435-1438 (1991); Xu et al., "The Role of  
Gibberellin, Absciscic Acid, and Sucrose in the Regulation of Potato Tuber  
Formation *In vitro*," Plant Physiol. 117:575-584 (1998), Sergeeva et al., "Tuber  
10 Morphology and Starch Accumulation are Independent Phenomena: Evidence  
from *ipt*-transgenic Potato Lines," Physiol. Plant 108:435-443 (2000)).  
Gibberellins (GA), in particular, play an important role in regulating tuber  
development. High levels of GA are correlated with the inhibition of tuberization,  
whereas low levels are associated with the induction of tuber formation (Jackson  
15 et al., "Control of Tuberisation in Potato by Gibberellins and Phytochrome," B.  
Physiol Plant 98:407-412 (1996); Xu et al., "The Role of Gibberellin, Absciscic  
Acid, and Sucrose in the Regulation of Potato Tuber Formation *In vitro*," Plant  
Physiol. 117:575-584 (1998)). Specific genes, such as lipoxygenases (Kolomiets  
et al., "Lipoxygenase is Involved in the Control of Potato Tuber Development,"  
20 Plant Cell 13:613-626 (2001)) and MADS box genes (Kang et al., "Nucleotide  
Sequences of Novel Potato MADS-box cDNAs and their Expression in vegetative  
Organs," Gene 166:329-330 (1995)) that are involved in regulating tuber  
formation have been identified.

[0012] Three independent research groups have recently confirmed that  
25 BEL-like TFs interact via protein binding with their respective *knox*-types in three  
separate species (Bellaoui et al., "The Arabidopsis BELL1 and KNOX TALE  
Homeodomain Proteins Interact Through a Domain Conserved Between Plants  
and Animals," Plant Cell 13:2455-2470 (2001); Müller et al., "*In vitro* Interactions  
Between Barley TALE Homeodomain Proteins Suggest a Role for Protein-Protein  
30 Associations in the Regulation of Knox Gene Function," Plant J. 27:13-23 (2001);  
Smith et al., "Selective Interaction of Plant Homeodomain Proteins Mediates High  
DNA-Binding Affinity," Proc. Nat'l. Acad. Sci. USA 99:9579-9584 (2002)), but

to date, there is no published report on the function of this interaction. Moreover, nothing is known about the role of either KNOX or the BEL TFs in the regulation of development of tuberous plants, such as potato.

[0013] The present invention is directed to overcoming these and other  
5 deficiencies in the art.

### SUMMARY OF THE INVENTION

[0014] The present invention relates to isolated nucleic acid molecules  
which encode a BEL transcription factor from potato (*Solanum tuberosum* L.) and  
10 the amino acid sequences encoded by such nucleic acid molecules.

[0015] Another aspect of the present invention pertains to host cells, DNA  
constructs, expression vectors, transgenic plants, and transgenic plant seeds  
containing the isolated nucleic acid molecules of the present invention.

[0016] The present invention is also directed to a method for enhancing  
15 tuber development in a plant. This method includes transforming a tuberous plant  
with a first DNA construct including a first nucleic acid molecule encoding a BEL  
transcription factor or a KNOX transcription factor, and a first operably linked  
promoter and first 3' regulatory region, whereby tuber development in the plant is  
enhanced.

20 [0017] A further aspect of the present invention relates to a method for  
enhancing growth in a plant. This method includes transforming a plant with a  
DNA construct including a nucleic acid molecule encoding a BEL transcription  
factor from *Solanum tuberosum* and an operably linked promoter and 3'  
regulatory region, whereby growth in the plant is enhanced.

25 [0018] Yet another aspect of the present invention relates to a method for  
regulating flowering in a plant. This method includes transforming a plant with a  
DNA construct including a nucleic acid molecule encoding a BEL transcription  
factor from *Solanum tuberosum* and an operably linked promoter and 3'  
regulatory region, whereby flowering in the plant is regulated.

[0019] The present invention relates to transcription factors which can be used to enhance tuber formation, to enhance growth, or to regulate flowering in a plant. In particular, accelerating tuber growth in field plants shortens the time for field cultivation. It can also be used to shorten the timing of a "late" potato  
5 variety to produce an earlier harvest. Many desirable breeding lines of potato produce tubers too late in the growing season or with too low a yield. The method of the present invention circumvents these problems, even under noninductive conditions. Enhanced tuberization also has applications for producing food in space under a research initiative directed by NASA (Food and Crop Systems  
10 Research, NASA's Advanced Life Support Program). Potato tubers are also being designed as biostorage organs for the production of pharmaceuticals or bioproducts. Enhanced tuber growth would be advantageous in these systems. Moreover, enhancement of growth in plants or regulation of flowering in plants can be used to produce an earlier harvest of plants/flowers.

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#### **BRIEF DESCRIPTION OF THE DRAWINGS**

[0020] Figure 1 shows Southern hybridization of *POTH1*. Genomic DNA (10 µg) was digested with the restriction enzymes, *Hind* III (H) or *Xba* I (X) and hybridized to a <sup>32</sup>P-labeled *POTH1* probe which did not include the ELK or  
20 homeodomain. There is a restriction site for *Hind* III within the coding sequence of *POTH1*. Size markers in kb are shown on the right.

[0021] Figure 2 shows *POTH1* mRNA accumulation in various organs of the potato plants. Poly(A)-enriched RNA (5 µg in each lane) was hybridized to a digoxigenin-rUTP-labeled *POTH1* RNA antisense probe with the ELK and  
25 homeodomain deleted. MT, mature tuber; S, stem; R, root; IN, inflorescence; ML, mature leaf; SA, shoot apex; SS, swollen stolon apex. Equal loading of intact poly(A)+ RNA in each lane was confirmed by ethidium bromide staining. The hybridizing bands are approximately 1.3 kb in length.

[0022] Figures 3A-F show the localization of *POTH1* mRNA in potato  
30 plants as revealed by *in situ* hybridization. The presence of *POTH1* mRNA is indicated by an orange/brown stain under dark-field microscopy. All micrographs

are of equal magnification. Size bar = 300  $\mu$ m. Figure 3A shows a longitudinal section through a vegetative shoot apex, probed with antisense *POTH1*. AP = apical meristem; L = leaf lamina; OL = older leaf lamina. Asterisks indicate leaf primordia (beneath AP) and procambium (to left of AP). Figure 3B shows  
5 unswollen stolon apex, antisense *POTH1*. AP = apical meristem; P = procambium; asterisk = lamina of young leaf; V = perimedullary parenchyma associated with vascular tissue; X = xylem element. Figure 3C shows unswollen stolon apex, sense *POTH1*. Figure 3D shows swollen stolon apex, antisense *POTH1*. AP = apical meristem; P = procambium; V = perimedullary parenchyma  
10 and vascular tissue; L = lamina of young leaf. Figure 3E shows swollen stolon, subapical longitudinal section, basal to section in 3D, antisense *POTH1*. IC = inner cortex; V = perimedullary parenchyma and vascular tissue; PI = pith. Figure 3F shows swollen stolon, subapical section, sense *POTH1*.

[0023] Figures 4A-F show *POTH1* mRNA accumulation in transgenic  
15 potato plants and the evaluation of leaf and stem traits in *POTH1* overexpression lines. Figure 4A shows total RNA (5  $\mu$ g) from shoot tips of wild-type (WT) and independent transgenic lines, potato subsp. *andigena* 15, 18, 20, 29, and 11 that were hybridized to a  $^{32}$ P-labeled *POTH1* probe with the ELK or homeodomain deleted. In Figure 4B, membranes were stripped and hybridized with  $^{32}$ P-labeled  
20 1.2 kb wheat 18S rRNA to ascertain equal loading and transfer. In Figures 4C-F, three plants each of wild-type and overexpression lines, potato subsp. *andigena* 15, 18, 20, 29, and 11 were examined. Standard error is indicated for each mean. In Figure 4C, plant height and in Figure 4D, internode length were examined for 75-day old plants. In Figure 4E, petiole length and in Figure 4F, the terminal  
25 leaflet length was measured for the sixth expanded leaf of 84-day old plants.

[0024] Figures 5A-Q show the phenotype of the leaves of *POTH1*  
overexpression lines. Figure 5A shows that the overall size and shape of leaves from the *andigena* intermediate and severe overexpression lines, line 20 and line  
15, respectively, have been altered compared to wild-type leaves (WT). In Figure  
30 5B, wild-type leaflets (WT) have a prominent mid-vein (mv) and pinnate venation pattern. The potato subsp. *andigena* intermediate overexpression mutant (line 20) has a mouse-ear shape, a shortened mid-vein, and palmate venation pattern. Figure



5C shows the shoot tip of WT potato subsp. *andigena* line. Figure 5D shows the severe mutant, potato subsp. *andigena* line 15, which has a mouse-ear leaf phenotype and shortened petioles causing leaves to cluster closely to the stem. The bars in Figures 5C and D are 5mm. In Figure 5E, the rachis and associated  
5 leaflets were detached from the petiole of a wild-type (WT) and a representative sense line (19), to show a slight increase in the proliferation of leaflets. Figure 5F shows a cross-section through a wild-type leaf showing the arrangement of cell layers: e = epidermis; sp = spongy parenchyma; pp = palisade parenchyma. Size bar = 50  $\mu$ m. Figure 5G shows a cross-section through a potato subsp. *andigena*  
10 line 15 leaf after treatment with GA<sub>3</sub> showing an intermediate level of cell organization. Bar = 50  $\mu$ m. Figure 5H shows a cross-section through a potato subsp. *andigena* line 15 leaf showing that the cell layers lack a palisade parenchyma layer. Size bar = 50  $\mu$ m. Figure 5I shows a wild-type leaf from potato subsp. *andigena* showing the morphology of a compound leaf. In Figures  
15 5J and K, the compound leaf structure is shown for the overexpression mutant, potato subsp. *andigena* line 15. Shoot tips were treated with either 10 $\mu$ M GA<sub>3</sub> in 0.002% (v/v) ethanol (Figure 5J) or with 0.002% (v/v) ethanol alone (Figure 5K). Terminal leaflets from compound leaves of wild-type plants (Figure 5L), GA<sub>3</sub>-treated line 15 (Figure 5M), and untreated line 15 (Figure 5N) are shown. The  
20 mid-vein is marked with an arrow in Figure 5M. Note that the morphology and venation of the GA<sub>3</sub>-treated leaf (Figures 5J and M) is more similar to the wild-type leaf (Figures 5I and L) than to the potato subsp. *andigena* line 15 untreated leaf (Figures 5K and N). Bars in Figures 5I through 5K = 1.0 mm. The second expanded leaf was used for the leaf samples in Figures 5F through 5N. Figure 5O  
25 is a wild-type leaf from *Solanum tuberosum* cv. FL-1607 ('FL-1607') showing the morphology of a compound leaf. In Figures 5P-Q, the compound leaf structure is shown for the overexpression mutant, 'FL-1607' line 5. Shoot tips were treated with either 10 $\mu$ M GA<sub>3</sub> in 0.002% (v/v) ethanol (Figure 5P) or with 0.002% (v/v) ethanol alone (Figure 5Q). The mid-vein is marked with an arrow in Figure 5P.  
30 Note that the morphology of the GA<sub>3</sub>-treated leaf (Figure 5P) is more similar to the wild-type leaf (Figure 5O) than to 'FL-1607' line 5 control leaf (Figure 5Q).

[0025] Figure 6 shows the levels of intermediates in the GA biosynthetic pathway. GAs were extracted from shoot tips down to the sixth expanded leaf from wild-type and potato subsp. *andigena* *POTH1* overexpression lines 29, 20, and 11. GAs were separated by HPLC and levels were measured by gas chromatography-mass spectrometry (GC-MS). GA<sub>53</sub>, GA<sub>19</sub>, and GA<sub>20</sub> are precursors to GA<sub>1</sub>, the physiologically active GA, whereas GA<sub>8</sub> is the inactive metabolite. GA<sub>53</sub> and GA<sub>19</sub> levels increased, whereas GA<sub>20</sub>, GA<sub>1</sub>, and GA<sub>8</sub> levels decreased in *POTH1* overexpression lines. Measurements are the average of three replications. Standard error is indicated for each mean. Concentrations of GA<sub>53</sub>, GA<sub>19</sub>, GA<sub>20</sub>, GA<sub>1</sub> and GA<sub>8</sub> were determined by calculating the area of the peaks at the correct Kovats retention indices (KRI) at 448/450 (KRI = 2,497), 434/436 (2,596), 418/420 (2,482), 506/508 (2,669), and 594/596 (2,818), respectively.

[0026] Figures 7A-B show the accumulation of mRNA for GA 20-oxidase1 in transgenic plants that overexpress the potato *knox* gene, *POTH1*. In Figure 7A, 5 µg of total RNA from the shoot tips of wild-type lines (designated 2, 9, and 10) and the overexpression lines, potato subsp. *andigena* 11, 15, and 18 were hybridized with a 1.2-kb fragment of the potato GA 20-oxidase1 cDNA, *StGA20ox1* (Carrera et al., "Feedback Control and Diurnal Regulation of Gibberellin 20-oxidase Transcript Levels in Potato," *Plant Physiol.* 119:765-773 (1999), which is hereby incorporated by reference in its entirety). In Figure 7B, the membrane was stripped and re-probed with 18S wheat rRNA to ascertain equal loading and efficient transfer.

[0027] Figure 8 shows GA 20-oxidase1 mRNA accumulation in shoot tips of *POTH1* overexpressers (plants #11, 15, and 18) with a severe phenotype (dwarf with small, curled leaves). Total RNA (10 µg in each lane) was hybridized to <sup>32</sup>P-labeled GA 20-oxidase1 (Carrera et al., "Feedback Control and Diurnal Regulation of Gibberellin 20-oxidase Transcript Level in Potato," *Plant Physiol.* 119:765-773 (1999), which is hereby incorporated by reference) probe. Standard procedures for RNA blot hybridization were used. The plants shown are 8 weeks old. These same plants had reduced levels of GA<sub>20</sub> and GA<sub>1</sub> and increased levels of GA<sub>53</sub> and GA<sub>19</sub>.

[0028] Figures 9A-C show the specific interaction of POTH1 with seven BEL1-like proteins of potato. Figure 9A shows selection on a nutrient carbon medium minus histidine, leucine, tryptophan, and adenine. The pAD plasmid provides leucine selection, the pBD plasmid (pBridge) provides tryptophan selection, and histidine and adenine selection are activated from the host strain (AH109) chromosomal DNA. The asterisk (\*) designation indicates yeast growth with both plasmids transformed together, whereas the pAD plasmids (designated 5, 11, 13, 14, 22, 29, 30) are transformed alone (no growth). SIR4, a transcriptional activator of yeast, is used as a positive control and pBHD is *POTH1* in pBridge alone. Figure 9B shows that POTH1 interacts with all seven BELs as determined by a quantitative yeast two-hybrid assay. *LacZ* induction in the yeast strain AH109 was assayed in transformed yeast cultures using a quantitative yeast  $\beta$ -galactosidase assay method (Pierce Chemical Company). For each pair, the dark bars on the left represent the pAD or pBHD plasmid alone transformed into yeast. The white bars on the right in each pair represent both plasmids (pAD and pBHD) transformed together. The standard error of the mean is represented by error bars. Figure 9C shows immunoprecipitates of the *in vitro* binding of POTH1 to BEL proteins of potato.  $^{35}\text{S}$ -labeled GAD: POTH1 fusion protein and the three BEL1 proteins (p11Z-5, -13, and -30) were synthesized in separate *in vitro* transcription/translation reactions (lanes 2, 3, 6, and 9, respectively). Each of the three BEL1 proteins were incubated with the GAD:POTH1 protein and immunoprecipitated with anti-GAD antibodies (lanes 5, 8, and 11). None of the three BEL proteins bound to the GAD protein alone (lanes 4, 7, and 10). Labeled proteins were visualized by autoradiography after separation by SDS-PAGE. Molecular size markers are shown on the right.

[0029] Figures 10A-B show a deletion analysis of the binding regions of POTH1 and a potato BEL1-like protein using the yeast two-hybrid system. In Figure 10A, deletion constructs of *POTH1* in pBridge were tested for expression in the yeast strain AH109 and cotransformed with the full-length BEL cDNA, *StBEL-05*, in pGAL4 to test for interaction. In Figure 10B, deletion constructs of *StBEL-05* in pGAL4 were cotransformed with the full-length cDNA of *POTH1* in pBridge. Interaction was verified with both nutritional selection and  $\beta$ -

galactosidase activity. The white box indicates the homeodomain. The gray box indicates the putative protein/protein interaction region (for POTH1, this is the conserved KNOX domain, for StBEL5, the BELL domain). The black boxes are conserved sequences identified in the BEL proteins (see Figure 13A) and the diagonal hatched boxes in POTH1 represent the ELK domain. The numbers in parentheses represent the amino acids of the full-length sequence included in each construct.

[0030] Figure 11A shows a Northern blot analysis of the accumulation of mRNA for four BEL1-like cDNAs (*StBEL-05*, *-13*, *-14*, and *-30*) in potato organs. Ten µg of total RNA from flowers, shoot tips (SAM), leaves, stems, roots, unswollen stolons (U stolon), swollen stolons (S stolon), and tubers were loaded per lane. Swollen stolons represent an early stage of tuber formation. A probe for the 18S ribosomal RNA was used to verify equal loading of RNA samples (bottom panel).

[0031] Figure 11B shows a Northern blot analysis of the accumulation of the mRNA of *StBEL-05* in leaves and stolons of WT plants grown under long days (LD, 16 hours of light, 8 hours of dark) and short days (SD, 8 hours of light, 16 hours of dark). Ten µg of total RNA from stolons were loaded per lane. Leaves and stolons were harvested from the photoperiod-responsive potato species, *Solanum tuberosum* ssp. *andigena*, 4 and 8 days after the plants were transferred to short-day conditions. Samples were harvested one hour after the end of the dark period. A gene-specific probe for each BEL cDNA was used. Ethidium bromide-stained ribosomal RNA is visualized as a loading control.

[0032] Figure 11C shows a Northern blot analysis of the accumulation of the mRNA of potato BEL-like cDNAs (*StBEL-05*, *StBEL-13*, *StBEL-14*, and *StBEL-30*) in tuberizing stolons. Ten µg of total RNA from stolons were loaded per lane. Stolons were harvested from the photoperiod-responsive potato species, *Solanum demissum*, 1, 2, 4, or 7 days after the plants were transferred to short-day conditions. A gene-specific probe for each BEL cDNA was used. A probe for the 18S ribosomal RNA was used to verify equal loading of RNA samples (bottom panels).

[0033] Figure 12 shows the phylogenetic tree of the BEL1-like proteins of potato (*Solanum tuberosum* L.). The amino acid sequence of seven potato BEL-like proteins was analyzed and compared to BEL proteins of plants. These data were organized into a phylogenetic tree with the ME-Boot program of the MEGA package (version 1.0) and the neighbor-joining program (Saitou and Nei, 1987). The numbers listed at the branching points are boot-strapping values which indicate the level of significance (%) for the separation of two branches. The length of the branch line indicates the extent of difference according to the scale at the lower left-hand side. Databank accession numbers are listed on the dendrogram and the common name of the species is listed in the right-hand column.

[0034] Figure 13A shows a schematic of the amino acid sequence of the BEL1-like proteins of potato. Boxed regions represent conserved sequences identified by aligning all seven BELs. Helices I, II, and III of the homeodomain are designated. The proline-tyrosine-proline (PYP) loop extension is located between helices I and II. For clarity in labeling, the sequence is not drawn to scale.

[0035] Figure 13B shows predicted helices of the putative protein-binding region (BELL domain) of the BEL1 protein StBEL-05. The bold letters represent amino acids conserved in other plant BEL1 proteins based on a BLAST analysis of StBEL-05. The underlined portion of the sequence represents a predicted  $\alpha$ -helix. A consensus for the prediction of the sequence structure was derived by using three software programs for amino acid sequence analysis: sspal, ssp, and nnssp (<http://www.softberry.com/protein.html>). Four deletion constructs from Figure 14B are designated with arrows. Construct pAD5-1 contains aa 230 through 653 of pAD-05 (interaction with POTH1), and pAD5-2 contains aa 257 through 653 of pAD-05 (no interaction). Construct pAD5-11 consists of aa 1 through 286 of pAD-05 (no interaction), and pAD5-9 consists of aa 1 through 315 (interaction with POTH1).

[0036] Figure 13C is a Southern blot analysis of BEL-like genes of potato. Genomic DNA (10  $\mu$ g per lane) was digested with EcoRI, HindIII, and PstI. Each

blot was hybridized with a  $^{32}\text{P}$ -labeled gene-specific probe from each of the four *StBEL* cDNAs. DNA size markers in kilobases are indicated on the right.

[0037] Figures 14A-C show *in vitro* tuberization of transgenic plants that overexpress sense transcripts of *StBEL-05*. Northern blot analysis for the accumulation of mRNA for *StBEL-05* was performed by using 10  $\mu\text{g}$  of total RNA/lane from vegetative meristems of *in vitro* plantlets and gene-specific probes for *StBEL-05* (see Figure 14A). Equal loading of RNA samples was verified by visualizing ethidium bromide-stained rRNA bands with UV light. The rate of tuberization (days to tuberize) was determined by the first appearance of tubers from among twenty-four replicates (see Figure 14B). The number of tubers was scored after 2 weeks of LD conditions (0 d), and after 7 (7 d) and 14 days (14 d) of SD conditions (see Figure 14B). Tubers were harvested and weighed after 21 days (see Figure 14C) from the *StBEL-05* overexpression (24 plants each) and wild-type lines (35 plants). Cultured transgenic plants of *Solanum tuberosum* ssp. *andigena* were grown on a Murashige and Skoog medium with 6 % sucrose under a long-day photoperiod (16 hours of light, 8 hours of dark) in a growth chamber for two weeks. For tuber induction, plants were transferred to a Murashige and Skoog medium supplemented with 6 % sucrose and evaluated daily for tuber formation under a short-day photoperiod (8 hours of light, 16 hours of dark) in the growth chamber until tubers formed. All numbered lines were verified as transgenic by using PCR with transgene-specific primers. Control plants were both nontransgenic (WT) and transgenic (*StBEL-05* line 6).

[0038] Figure 15 shows overexpression mutant lines for the potato KNOX gene, *POTH1* (lines 15 and 18), and for the BEL1-like protein, *StBEL-05* (lines 12, 14, and 19). These *StBEL-05* sense lines had a leaf phenotype similar to wild-type plants (WT). These are 8-week plants grown under long-day conditions (16 hours of light, 8 hours of dark) in the greenhouse supplemented with high pressure sodium HID lamps. The *StBEL-05* plants ranged in height from 34 to 39 cm, whereas, the *POTH1* lines were 7 to 10 cm in height.

[0039] Figures 16A-B are a Northern blot analysis of the accumulation of the mRNA of the GA 20-oxidase1 gene of potato (Carerra et al., "Feedback Control and Diurnal Regulation of Gibberellin 20-oxidase Transcript Levels in

Potato," Plant Physiol. 119:765-773 (1999), which is hereby incorporated by reference in its entirety) in wild-type plants and sense lines 11, 12, and 20 of *StBEL-05* (Figure 16A). Total RNA was extracted from the 2.0 mm distal tip of stolons from plants grown under LD conditions (16 hours of light, 8 hours of dark). Wild-type RNA (WT) was extracted from two separate pools. Ten µg of total RNA were loaded per lane. A gene-specific probe for GA 20-oxidase1 was used for hybridization. All three *StBEL-05* lines exhibited enhanced tuber formation. Ethidium bromide-stained rRNA is visualized as a loading control (Figure 16B).

10 [0040] Figure 17A shows tubers harvested from independent lines of *StBEL-05* transgenic plants (*Solanum tuberosum* spp. *andigena*) grown in soil under a short-day photoperiod. Plants were grown under long days (LD) (16 hours of light, 8 hours of dark) in 10 cm pots until they reached the 16-leaf stage and then transferred to short days. After 14 days under short days, tubers from 15 three plants per independent line were harvested and photodocumented. Tuber numbers and yields increased by at least threefold in these *StBEL-05* lines relative to control plants. Starting from the upper left-hand corner and proceeding clockwise are tubers harvested from control plants (WT) and from each of the *StBEL-05* overexpression lines 14, 19, and 12. Other than the increase in the rate of tuber formation, the phenotype of these sense lines was similar to wild-type. 20 Reference bar is equivalent to 1.0 cm.

[0041] Figure 17B shows tubers from the same *StBEL-05* lines from Figure 17A harvested after 21 days of culture *in vitro* under inductive conditions of a short-day photoperiod (8 hours of light, 16 hours of dark) and 6 % sucrose in 25 the media. Tubers from 35 control plants and from 25 plants of the *StBEL-05* lines are displayed in the same order as shown in Figure 17A. Tuber yield per plant of line 14 was sixteenfold greater than wild-type. The tubers showed an intense purple color, which is the result of anthocyanin accumulation characteristic of this subspecies. Reference bar is equivalent to 1.0 cm.

30 [0042] Figure 17C shows tuber production for stolons from overexpression lines of POTH1. Excised stolon tips from plants grown under LD conditions were grown *in vitro* in the dark in media supplemented with 8 %

sucrose. Tubers were harvested after 35 days of culture. Starting from the upper left-hand corner and proceeding clockwise are tubers harvested from control plants (WT) and from each of the POTH1 overexpression lines 11, 18, and 20. Twelve stolon tips per independent line were evaluated for tuber production.

5 Reference bar is equivalent to 1.0 cm.

[0043] Figure 17D shows the rate of tuberization for stolons from overexpression lines 11, 18, 20, 29, and 15 of POTH1 and from wild-type plants (WT). Excised stolon tips (approximately 1.5 cm in length) from plants grown under long-day conditions were grown *in vitro* in the dark in media supplemented with 8% (w/v) sucrose and monitored for 20 days.

[0044] Figures 18A-B show gel mobility shift assays (Figure 18A) for the binding of two transcription factors of potato, POTH1 (HD) and StBEL-05, to regions of the GA20 oxidase1 promoter and the first intron (Figure 18B). Each DNA probe is tested for binding in four sets: DNA alone, with StBEL-05 only, with POTH1 (HD) only, and with both StBEL-05 and POTH1. The two proteins appear to bind in tandem to the P1 region. Two-hundred ng of purified protein and <sup>32</sup>P-labeled DNA fragments were used in each binding reaction. The protein/DNA mix was run on a nondenaturing polyacrylamide gel. These results are representative of several replications. The GA20 ox1 promoter was provided by Salomé Prat, Barcelona.

[0045] Figure 19 shows the effect of binding two transcription factors to the GA20 oxidase1 promoter on the rate of transcription. The potato GA20 oxidase1 promoter (1170 bp) plus an enhancer was fused to a GUS marker (GAPGUS, gray bars). The two transcription factors, POTH1 and StBEL-05, were cloned and expressed in separate protein expression vectors. All constructs were transformed into tobacco protoplasts through electroporation. Whereas, repression of transcription was affected by each TF alone, expression of the proteins in tandem resulted in the greatest repression of transcription. Activity of the 35SGUS construct (black bars) was used as a baseline control. The "no protein" protoplasts are designated as 100% transcriptional activity. All activities are calculated in relation to a luciferase internal control.



[0046] Figure 20 shows GA20 oxidase1 mRNA accumulation in stolon tips of plants grown under long-day conditions. Ten µg of total RNA was probed with a <sup>32</sup>P-fragment specific for the potato GA20 oxidase1 cDNA. These StBEL-05 lines all exhibited enhanced tuber formation.

5 [0047] Figures 21A-B show a competition gel-retardation assay of P1 with cold P1 or P3 in the presence of StBEL-05 (Figure 21A) or POTH1 (Figure 21B). Lane 1 is labeled P1 alone, lane 2 is the labeled P1 with either StBEL-05 (Figure 21A) or POTH1 (Figure 21B). Increased amounts (10X, 25X, 50X, 100X) of unlabeled P1 or P3 were added to lanes 3 to 6 and 7 to 10, respectively. The  
10 DNA-protein complexes are indicated with arrowheads.

[0048] Figure 22 shows a dissociation rate analysis of StBEL-05-P1, POTH1-P1, and StBEL-05-POTH1-P1 complexes. Labeled P1 was incubated on ice for 30 minutes with recombinant proteins, as indicated on the top. Then a 500-fold molar excess of unlabeled P1 was added and aliquots analyzed by gel  
15 mobility shift assay after the indicated time. The arrows show the DNA-protein complexes.

[0049] Figures 23A-B show the protein structures of POTH1 (Figure 23A) and StBEL-05 (Figure 23B). Conserved regions are labeled. These include the protein-binding regions for POTH1, KNOX I and KNOX II, and for StBEL-05,  
20 the Sky box and the BELL domains. The DNA-binding domains (HD) consisting of three helices and the characteristic proline-tyrosine-proline TALE are also designated. POTH1 is 345 aa in length, whereas StBEL-05 is 688 aa. The schematics of protein structure presented here are not drawn to scale to enhance visual clarity.

25 [0050] Figures 24A-C show schematics of constructs (Figure 24A) and the repression effect of StBEL-05 and POTH1 on the *ga20ox1* promoter (Figure 24B) and on the 35S CaMV promoter (Figure 24C). The construct with the LUC gene under the control of the cauliflower mosaic virus (CaMV) 35S promoter was used as an internal control. Each transfection was performed three times. Relative  
30 GUS-LUC activity was calculated with reporter alone set as 100%. Data are means ± SE.

[0051] Figures 25 A-C show schematics of constructs (Figure 25A) and the effect of dominant negative constructs of either StBEL-05 or POTH1 on the repression activity of StBEL-05 (Figure 25B) or POTH1 (Figure 25C), respectively. The construct with the LUC gene under the CaMV 35S promoter was used as a control. Each transfection was performed three times. Relative GUS-LUC activity was calculated with reporter alone set as 100%. Data are means  $\pm$  SE.

[0052] Figures 26A-C show a schematic of the mutated base in a 9-bp motif (Figure 26A) and that mutation in the StBEL-05-POTH1 heterodimer binding site deprived the ga20ox1 promoter of its response to StBEL-05 and POTH1 repression (Figures 26B-C). The construct with the LUC gene under the CaMV 35S promoter was used as control. Each transfection was performed three times. Relative GUS-LUC activity was calculated with reporter alone set as 100%. Data are means  $\pm$  SE.

[0053] Figure 27 shows a model of BEL/KNOX binding to target DNA. Light grey = StBEL-05 homeodomain; dark grey = POTH1 homeodomain. The three helices are indicated as I, II, or III. The schematics of protein structure presented here are not drawn to scale to enhance visual clarity. The third helix of the homeodomains of both POTH1 and StBEL-05 fit in the major groove of the DNA double helix.

### **DETAILED DESCRIPTION OF THE INVENTION**

[0054] The present invention relates to nucleic acid molecules encoding BEL transcription factors from potato (*Solanum tuberosum* L.). BEL transcription factor is a general term used herein to mean a member of the *BEL-1*-like family of transcription factors, which includes a BELL domain (Bellaoui et al., "The Arabidopsis BELL1 and KNOX TALE Homeodomain Proteins Interact Through a Domain Conserved Between Plants and Animals," Plant Cell 13(11):2455-70 (2001), which is hereby incorporated by reference in its entirety) and which regulates growth, in particular, floral development.

[0055] In a first embodiment, the BEL transcription factor from *Solanum tuberosum* is identified herein as StBEL-05 and is encoded by a nucleic acid molecule having a nucleotide sequence of SEQ ID NO:1 as follows:

```

5      1 catgcagaga taaaaatata gatcagtctg acaagaaggc aactttctca agcttagaga
      61 gctaccaccc gaagatagac agttagttac atgtactgtt atagataaaa ggagaaatcc
     121 gaagaagaaa gaattttttt tgcagatatg tactatcaag gaacctcgga taataactaat
     181 atacaagctg atcatcaaca acgtcataat catgggaata gtaataataa taatattcag
    241 acactttatt tgatgaaccc taacaattat atgcaaggct acactacttc tgacacacag
    301 cagcagcagc agttactttt cctgaattct tcaccagcag caagcaacgc gctttgccat
    361 gcgaatatac aacacgcgcc gctgcaacag cagcactttg tcggtgtgcc tcttcgccga
    421 gtaagtttgc acgatcagat caatcatcat ggacttttac agcgcatgtg gaacaaccaa
    481 gatcaatctc agcaggtgat agtaccatcg tcgacggggg tttctgccac gtcagtgtggc
    541 gggatcacca cggactttgg gtctcaattg gcgtttcaga ggccgattcc gacaccacaa
    601 caccgacagc agcaacaaca gcaaggcggg ctatctctaa gccttttccc tcagctacaa
    661 cagcaaatga gtttcaataa caatatttca tcctcatcac caaggacaaa taatgttact
    721 attaggggaa cattagatgg aagttctagc aacatggttt taggctctaa gtatctgaaa
    781 gctgcacaag agcttcttga tgaagttggt aatattgttg gaaaaagcat caaaggagat
    841 gatcaaaaga aggataattc aatgaataaa gaatcaatgc ctttggctag tgatgtcaac
    901 actaatagtt ctggtggtgg tgaaagtagc agcaggcaga aaaaatgaag tgcgtgtgag
    961 cttacaactg ctcaaagaca agaacttcaa atgaaaaaag ccaagcttct tgccatgctt
   1021 gaagagggtg agcaaaggta cagacagtac catcaccaaa tgcaataaat tgtattatca
   1081 tttgagcaag tagcaggaat tggatcagcc aaatcataca ctcaattagc tttgcatgca
   1141 atttcgaagc aattcagatg cctaaaggat gcaattgctg agcaagtaaa ggcgacgagc
   1201 aagagtttag gtgaagagga aggccttgga gggaaaaatc aaggctcaag actcaaatct
   1261 gtggaccatc atctaaggca acaacgcgcg ctgcaacaga taggaatgat gcaaccaaat
   1321 gcttgagac ccctaaaggg tttacctgaa agagctgtct ctgtccttcg tgcttgctt
   1381 ttcgagcatt ttcttcatcc ttaccctaaag gattcagaca aaatcatgct tgctaagcaa
   1441 acggggctaa caaggagcca ggtgtctaac tggttcataa atgctcgagt tcgattatgg
   1501 aagccaatgg tagaagaaat gtacttggaa gaagtgaaga atcaagaaca aaacagtact
   1561 aatacttcag gagataacaa aaacaaagag accaatataa gtgctccaaa tgaagagaaa
   1621 catccaatta ttactagcag cttattacaa gatggtatta ctactactca agcagaaaat
   1681 tctacctcaa ctatttcaac ttcccctact gcagggtgctt cacttcatca tgctcacaat
   1741 ttctccttcc ttggttcatt caaatggat aatactacta ctactgttga tcatattgaa
   1801 aacaacgcga aaaagcaaaag aaatgacatg cacaagtttt ctccaagtag tattctttca
   1861 tctgttgaca tggaaagcaa agctagagaa tcatcaataa aagggtttac taatccttta
   1921 atggcagcat acgcgatggg agatttttga aggtttgatc ctcatgatca acaaatgacc
   1981 gcgaattttc atggaaataa tgggtgtctc ctacttttag gacttcctcc ttctgaaaac
   2041 ctagccatgc cagtgaagca acaaaattac ctttctaag acttgggaag taggtctgaa
   2101 atggggagtc attacaatag aatgggatat gaaaacattg attttcagag tgggaataag
   2161 cgattttccg ctcaactatt accagatttt gttacaggta atctaggaac atgaatacca
   2221 gaaagtctcg tattgatagc tgaagagata aaaggaagtt agggatactc ttatattgtg
   2281 tgaggccttc tggcccaagt cggaggaccc aatttgatac aacctatcat aggagaaaag
   2341 aagtggagac taaattaaag taacaaaatt ttaaagcaca ctttctagta tatatacttc
   2401 ttttttttat agtatagaaa agaagagatt ttgtgtctta gtgtatagat agagctact
   2461 tagtataggt tatacttcta gttccttgag aagattgata caactagtag tatttttttt
   2521 cttttgggtt ggcttgagat actattttta gttattggaa actagctata gtaaatgttg
   2581 taaagttgtg atattgttcc tctcaatttg catataattt gaaatatttt gtacctacta
   2641 gctagtctct aaattatgtt tccattgctt gtaattgcaa ttttatttga attttgtgct
   2701 atcattatta gattagcaaa aaaaaaaaaa aaaaa

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[0056] The nucleic acid sequence corresponding to SEQ ID NO:1 encodes a BEL transcription factor isolated from *Solanum tuberosum* identified herein as StBEL-05, which has a deduced amino acid sequence corresponding to SEQ ID

NO:2 as follows:

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Met Tyr Tyr Gln Gly Thr Ser Asp Asn Thr Asn Ile Gln Ala Asp His
  1                      5                      10                      15
60 Gln Gln Arg His Asn His Gly Asn Ser Asn Asn Asn Asn Ile Gln Thr
    20                      25                      30

```

	Leu	Tyr	Leu	Met	Asn	Pro	Asn	Asn	Tyr	Met	Gln	Gly	Tyr	Thr	Thr	Ser
			35					40					45			
5	Asp	Thr	Gln	Gln	Gln	Gln	Gln	Leu	Leu	Phe	Leu	Asn	Ser	Ser	Pro	Ala
		50					55					60				
	Ala	Ser	Asn	Ala	Leu	Cys	His	Ala	Asn	Ile	Gln	His	Ala	Pro	Leu	Gln
	65					70					75					80
10	Gln	Gln	His	Phe	Val	Gly	Val	Pro	Leu	Pro	Ala	Val	Ser	Leu	His	Asp
					85					90					95	
	Gln	Ile	Asn	His	His	Gly	Leu	Leu	Gln	Arg	Met	Trp	Asn	Asn	Gln	Asp
15				100					105					110		
	Gln	Ser	Gln	Gln	Val	Ile	Val	Pro	Ser	Ser	Thr	Gly	Val	Ser	Ala	Thr
			115					120					125			
20	Ser	Cys	Gly	Gly	Ile	Thr	Thr	Asp	Leu	Ala	Ser	Gln	Leu	Ala	Phe	Gln
		130					135					140				
	Arg	Pro	Ile	Pro	Thr	Pro	Gln	His	Arg	Gln	Gln	Gln	Gln	Gln	Gln	Gly
	145					150					155					160
25	Gly	Leu	Ser	Leu	Ser	Leu	Ser	Pro	Gln	Leu	Gln	Gln	Gln	Ile	Ser	Phe
					165					170					175	
	Asn	Asn	Asn	Ile	Ser	Ser	Ser	Ser	Pro	Arg	Thr	Asn	Asn	Val	Thr	Ile
30				180					185					190		
	Arg	Gly	Thr	Leu	Asp	Gly	Ser	Ser	Ser	Asn	Met	Val	Leu	Gly	Ser	Lys
			195					200					205			
35	Tyr	Leu	Lys	Ala	Ala	Gln	Glu	Leu	Leu	Asp	Glu	Val	Val	Asn	Ile	Val
		210					215					220				
	Gly	Lys	Ser	Ile	Lys	Gly	Asp	Asp	Gln	Lys	Lys	Asp	Asn	Ser	Met	Asn
	225					230					235					240
40	Lys	Glu	Ser	Met	Pro	Leu	Ala	Ser	Asp	Val	Asn	Thr	Asn	Ser	Ser	Gly
					245					250					255	
	Gly	Gly	Glu	Ser	Ser	Ser	Arg	Gln	Lys	Asn	Glu	Val	Ala	Val	Glu	Leu
45				260					265					270		
	Thr	Thr	Ala	Gln	Arg	Gln	Glu	Leu	Gln	Met	Lys	Lys	Ala	Lys	Leu	Leu
			275					280					285			
50	Ala	Met	Leu	Glu	Glu	Val	Glu	Gln	Arg	Tyr	Arg	Gln	Tyr	His	His	Gln
		290					295					300				
	Met	Gln	Ile	Ile	Val	Leu	Ser	Phe	Glu	Gln	Val	Ala	Gly	Ile	Gly	Ser
	305					310					315					320
55	Ala	Lys	Ser	Tyr	Thr	Gln	Leu	Ala	Leu	His	Ala	Ile	Ser	Lys	Gln	Phe
					325					330					335	
	Arg	Cys	Leu	Lys	Asp	Ala	Ile	Ala	Glu	Gln	Val	Lys	Ala	Thr	Ser	Lys
60				340					345					350		

	Ser	Leu	Gly	Glu	Glu	Glu	Gly	Leu	Gly	Gly	Lys	Ile	Glu	Gly	Ser	Arg	
			355					360					365				
5	Leu	Lys	Phe	Val	Asp	His	His	Leu	Arg	Gln	Gln	Arg	Ala	Leu	Gln	Gln	
		370					375					380					
	Ile	Gly	Met	Met	Gln	Pro	Asn	Ala	Trp	Arg	Pro	Gln	Arg	Gly	Leu	Pro	
10		385				390					395				400		
	Glu	Arg	Ala	Val	Ser	Val	Leu	Arg	Ala	Trp	Leu	Phe	Glu	His	Phe	Leu	
					405					410					415		
15	His	Pro	Tyr	Pro	Lys	Asp	Ser	Asp	Lys	Ile	Met	Leu	Ala	Lys	Gln	Thr	
				420					425					430			
	Gly	Leu	Thr	Arg	Ser	Gln	Val	Ser	Asn	Trp	Phe	Ile	Asn	Ala	Arg	Val	
			435					440					445				
20	Arg	Leu	Trp	Lys	Pro	Met	Val	Glu	Glu	Met	Tyr	Leu	Glu	Glu	Val	Lys	
		450					455					460					
	Asn	Gln	Glu	Gln	Asn	Ser	Thr	Asn	Thr	Ser	Gly	Asp	Asn	Lys	Asn	Lys	
25		465				470					475				480		
	Glu	Thr	Asn	Ile	Ser	Ala	Pro	Asn	Glu	Glu	Lys	His	Pro	Ile	Ile	Thr	
					485					490					495		
30	Ser	Ser	Leu	Leu	Gln	Asp	Gly	Ile	Thr	Thr	Thr	Gln	Ala	Glu	Ile	Ser	
			500					505						510			
	Thr	Ser	Thr	Ile	Ser	Thr	Ser	Pro	Thr	Ala	Gly	Ala	Ser	Leu	His	His	
			515					520					525				
35	Ala	His	Asn	Phe	Ser	Phe	Leu	Gly	Ser	Phe	Asn	Met	Asp	Asn	Thr	Thr	
		530					535					540					
	Thr	Thr	Val	Asp	His	Ile	Glu	Asn	Asn	Ala	Lys	Lys	Gln	Arg	Asn	Asp	
40		545				550					555				560		
	Met	His	Lys	Phe	Ser	Pro	Ser	Ser	Ile	Leu	Ser	Ser	Val	Asp	Met	Glu	
					565					570				575			
45	Ala	Lys	Ala	Arg	Glu	Ser	Ser	Asn	Lys	Gly	Phe	Thr	Asn	Pro	Leu	Met	
			580						585					590			
	Ala	Ala	Tyr	Ala	Met	Gly	Asp	Phe	Gly	Arg	Phe	Asp	Pro	His	Asp	Gln	
			595					600					605				
50	Gln	Met	Thr	Ala	Asn	Phe	His	Gly	Asn	Asn	Gly	Val	Ser	Leu	Thr	Leu	
		610					615					620					
	Gly	Leu	Pro	Pro	Ser	Glu	Asn	Leu	Ala	Met	Pro	Val	Ser	Gln	Gln	Asn	
55		625				630					635				640		
	Tyr	Leu	Ser	Asn	Asp	Leu	Gly	Ser	Arg	Ser	Glu	Met	Gly	Ser	His	Tyr	
					645					650				655			
60	Asn	Arg	Met	Gly	Tyr	Glu	Asn	Ile	Asp	Phe	Gln	Ser	Gly	Asn	Lys	Arg	
				660					665					670			

Phe Pro Thr Gln Leu Leu Pro Asp Phe Val Thr Gly Asn Leu Gly Thr  
675 680 685

5

The BEL transcription factor has a molecular mass of approximately 75.7 kDa. *StBEL-05*, isolated from *Solanum tuberosum*, has a single open reading frame ("ORF") of 2067 bp, extending between nucleotides 148-2214.

[0057] In a second embodiment, the BEL transcription factor from  
10 *Solanum tuberosum* is identified herein as StBEL-11 and is encoded by a nucleic acid molecule having a nucleotide sequence of SEQ ID NO:3 as follows:

1 atgactttca ggtctagtct tccactagac ctccgtgaaa tttcaacaac aaatcatcaa  
61 gttggaatac tatcatcatc accattacca tcaccaggaa caaataccaa taatatcaat  
15 121 catactcgag gattaggggc atcatcatct ttttcgattt ctaatgggat gatattgggt  
181 tctaagtacc taaaagttgc acaagatctt cttgatgaag ttgttaatgt tggaaaaaac  
241 atcaaattat cagatggctt agagagtggg gcaaaggaga aacacaaatt ggacaatgaa  
301 ttaatatctt tggctagtga tgatgttgaa agcagcagcc aaaaaaatag tgggtgtgaa  
361 cttacaacag ctcaaagaca agaacttcaa atgaagaaag ccaagcttgt tagcatgctt  
20 421 gatgaggtgg atcaaaggta tagacaatac catcaccaaa tgcaaatgat tgcaacatca  
481 tttgagcaaa caacaggaat tggatcatca aaatcataca cacaacttgc tttgcacaca  
541 atttcaaagc aatttagatg tttaaaagat gcaatttctg ggcaaaataa ggacactagc  
601 aaaacttttag gggaagaaga aaacattgga ggcaaaattg aaggatcaaa gttgaaattt  
25 661 gtggatcatc atttacgcca acaacgtgca ctacaacaat tagggatgat gcaaaccaat  
721 gcattggaagc ctcaaagagg tttgccagaa agagcggttt cagttctccg cgcttggtt  
781 ttcgagcatt ttcttcatcc gtatcccaaa gattcagata aaatcatcct tgctaagcaa  
841 acagggctaa caaggagcca ggtatcaaat tgggtttataa atgctagagt tagactatgg  
901 aagccaatgg tagaagaaat gtacatggaa gaagtgaaga aaaacaatca agaacaaaat  
961 attgagccta ataacaatga aattgttggc tcaaaatcaa gtgttccaca agagaaatta  
30 1021 ccaatttagta gcaatattat tcataatgct tctccaaatg atatttctac ttccaccatt  
1081 tcaacatctc cgacgggtgg cgcggttctg attccgactc agacggttgc aggtttctcc  
1141 ttcattaggt cattaaacat ggagaacatt gatgatcaaa ggaacaacaa aaaggcaaga  
1201 aatgagatgc aaaattgttc aactagtact attctctcaa tggaaagaga aatcataaat  
35 1261 aaagtgtgac aagatgagac aatcaaaagt gaaaagttca acaacacaca aacaagagaa  
1321 tgttactctc taatgactcc aaattacaca atggatgatc aatttggaaac aaggttcaat  
1381 aatcaaaaatc atgaacaatt ggcaacaaca acaacttttc atcaaggaaa tggatcatgtt  
1441 tctcttactt tagggcttcc accaaattct gaaaaccaac acaattacat tggattggaa  
1501 aatcattaca atcaacctac acatcatcca aatattagct atgaaaacat tgattttcag  
1561 agtggaagac gatacgccac tcaactatta caagattttg tttcttgatg atatatataa  
40 1621 tttgcaggta aatcagcttg aaattacatc atgacaggtc ttgaataaaa gaaggggagt  
1681 tgagatttag tgatcatata aatatgtata ggtagaaatt ttagtttagta tatataggtt  
1741 atacttctag tttcttaatg aagatacaag ttttgtgtgt atttttgtat tgaggtaact  
1801 agctagcttg gattatttaa agttgtgtga tgcaactaaa gaagaagaaa aaataatcta  
45 1861 tatatgcaaa ctacagtata ttgtaaattt tgtgcttc

[0058] The nucleic acid sequence corresponding to SEQ ID NO:3 encodes  
a BEL transcription factor isolated from *Solanum tuberosum* identified herein as  
StBEL-11, which has a deduced amino acid sequence corresponding to SEQ ID  
NO:4 as follows:

50

Met Thr Phe Arg Ser Ser Leu Pro Leu Asp Leu Arg Glu Ile Ser Thr  
1 5 10 15

	Thr	Asn	His	Gln	Val	Gly	Ile	Leu	Ser	Ser	Ser	Pro	Leu	Pro	Ser	Pro	
				20					25					30			
5	Gly	Thr	Asn	Thr	Asn	Asn	Ile	Asn	His	Thr	Arg	Gly	Leu	Gly	Ala	Ser	
			35					40					45				
	Ser	Ser	Phe	Ser	Ile	Ser	Asn	Gly	Met	Ile	Leu	Gly	Ser	Lys	Tyr	Leu	
		50					55					60					
10	Lys	Val	Ala	Gln	Asp	Leu	Leu	Asp	Glu	Val	Val	Asn	Val	Gly	Lys	Asn	
	65					70					75					80	
	Ile	Lys	Leu	Ser	Asp	Gly	Leu	Glu	Ser	Gly	Ala	Lys	Glu	Lys	His	Lys	
15					85					90					95		
	Leu	Asp	Asn	Glu	Leu	Ile	Ser	Leu	Ala	Ser	Asp	Asp	Val	Glu	Ser	Ser	
				100					105					110			
20	Ser	Gln	Lys	Asn	Ser	Gly	Val	Glu	Leu	Thr	Thr	Ala	Gln	Arg	Gln	Glu	
			115					120					125				
	Leu	Gln	Met	Lys	Lys	Ala	Lys	Leu	Val	Ser	Met	Leu	Asp	Glu	Val	Asp	
25		130					135					140					
	Gln	Arg	Tyr	Arg	Gln	Tyr	His	His	Gln	Met	Gln	Met	Ile	Ala	Thr	Ser	
	145					150					155					160	
	Phe	Glu	Gln	Thr	Thr	Gly	Ile	Gly	Ser	Ser	Lys	Ser	Tyr	Thr	Gln	Leu	
30					165					170					175		
	Ala	Leu	His	Thr	Ile	Ser	Lys	Gln	Phe	Arg	Cys	Leu	Lys	Asp	Ala	Ile	
				180					185					190			
35	Ser	Gly	Gln	Ile	Lys	Asp	Thr	Ser	Lys	Thr	Leu	Gly	Glu	Glu	Glu	Asn	
			195					200					205				
	Ile	Gly	Gly	Lys	Ile	Glu	Gly	Ser	Lys	Leu	Lys	Phe	Val	Asp	His	His	
40		210					215					220					
	Leu	Arg	Gln	Gln	Arg	Ala	Leu	Gln	Gln	Leu	Gly	Met	Met	Gln	Thr	Asn	
	225					230					235					240	
	Ala	Trp	Lys	Pro	Gln	Arg	Gly	Leu	Pro	Glu	Arg	Ala	Val	Ser	Val	Leu	
45					245					250					255		
	Arg	Ala	Trp	Leu	Phe	Glu	His	Phe	Leu	His	Pro	Tyr	Pro	Lys	Asp	Ser	
				260					265					270			
50	Asp	Lys	Ile	Ile	Leu	Ala	Lys	Gln	Thr	Gly	Leu	Thr	Arg	Ser	Gln	Val	
			275					280					285				
	Ser	Asn	Trp	Phe	Ile	Asn	Ala	Arg	Val	Arg	Leu	Trp	Lys	Pro	Met	Val	
55		290					295					300					
	Glu	Glu	Met	Tyr	Met	Glu	Glu	Val	Lys	Lys	Asn	Asn	Gln	Glu	Gln	Asn	
	305					310					315					320	
	Ile	Glu	Pro	Asn	Asn	Asn	Glu	Ile	Val	Gly	Ser	Lys	Ser	Ser	Val	Pro	
60					325					330					335		

	Gln	Glu	Lys	Leu	Pro	Ile	Ser	Ser	Asn	Ile	Ile	His	Asn	Ala	Ser	Pro	
				340					345					350			
5	Asn	Asp	Ile	Ser	Thr	Ser	Thr	Ile	Ser	Thr	Ser	Pro	Thr	Gly	Gly	Gly	
			355					360					365				
	Gly	Ser	Ile	Pro	Thr	Gln	Thr	Val	Ala	Gly	Phe	Ser	Phe	Ile	Arg	Ser	
		370					375					380					
10	Leu	Asn	Met	Glu	Asn	Ile	Asp	Asp	Gln	Arg	Asn	Asn	Lys	Lys	Ala	Arg	
	385					390					395					400	
	Asn	Glu	Met	Gln	Asn	Cys	Ser	Thr	Ser	Thr	Ile	Leu	Ser	Met	Glu	Arg	
15					405					410					415		
	Glu	Ile	Ile	Asn	Lys	Val	Val	Gln	Asp	Glu	Thr	Ile	Lys	Ser	Glu	Lys	
				420					425					430			
20	Phe	Asn	Asn	Thr	Gln	Thr	Arg	Glu	Cys	Tyr	Ser	Leu	Met	Thr	Pro	Asn	
		435						440					445				
	Tyr	Thr	Met	Asp	Asp	Gln	Phe	Gly	Thr	Arg	Phe	Asn	Asn	Gln	Asn	His	
25		450				455						460					
	Glu	Gln	Leu	Ala	Thr	Thr	Thr	Phe	His	Gln	Gly	Asn	Gly	His	Val		
	465					470				475					480		
	Ser	Leu	Thr	Leu	Gly	Leu	Pro	Pro	Asn	Ser	Glu	Asn	Gln	His	Asn	Tyr	
30					485					490					495		
	Ile	Gly	Leu	Glu	Asn	His	Tyr	Asn	Gln	Pro	Thr	His	His	Pro	Asn	Ile	
				500					505					510			
35	Ser	Tyr	Glu	Asn	Ile	Asp	Phe	Gln	Ser	Gly	Lys	Arg	Tyr	Ala	Thr	Gln	
		515						520					525				
	Leu	Leu	Gln	Asp	Phe	Val	Ser										
40		530					535										

The BEL transcription factor has a molecular mass of approximately 59 kDa. *StBEL-11*, isolated from *Solanum tuberosum*, has a single open reading frame ("ORF") of 1608 bp, extending between nucleotides 1-1608.

45 [0059] In a third embodiment, the BEL transcription factor from *Solanum tuberosum* is identified herein as StBEL-13 and is encoded by a nucleic acid molecule having a nucleotide sequence of SEQ ID NO:5 as follows:

50	1	ggggagcgag	tggttccgac	aaggtatggt	aatgggtgga	gggtgcaagta
	51	gtcaacaatt	gggatatgca	aaaaatcata	ctcctaattgt	ggcggagtcc
	101	atgcaacttt	ttctaataagaa	tccacaacca	aggtcacctt	ctccatctcc
	151	tcctaattca	acttcttcta	cgtttcacat	gttggttacca	aacctcatcat



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201 ctacttcaac acttcaaggg tttcctaata cggccgaagg atctttcggg
251 caattcatta catgggggaa tggaggagca agtgctgcca cagccaccca
301 tcatctcaat gcccagaatg aaatcggagg agtaaactgt gtagaaagtc
351 aaggcctatc tctatccttg tcttcttcgt tacagcaca ggcggaggaa
5 401 ttacaaatga gcggagaagc tggagggaat atgttcttca atcaaggagg
451 gtctagtact tccgggcagt atcgatacaa gaatttgaat atgggtggat
501 caggagtaag cccaaacatt catcaagtcc atgttgggta tgggtcatca
551 ttaggagtgg tcaatgtgtt gaggaattcc aaatgcagat gggttgaaat
10 601 agaactactg gaagaattct gcagtgttgg aagaggtaaa ttgaagaaga
651 ctaacaacaa agcagcagcc aataacccta atacgaaccc tagtggcgct
701 aacaatgaag cttcttcaaa agatgttcct actttgtccg ctgctgatag
751 aattgagcat cagagaagga aggtcaaact tttatctatg gttgatgagg
801 tagataggag gtacaatcat tactgtgaac aaatgcagat gggttgaaat
15 851 tcgtttgatt tagtgatggg tttcggcaca gcagttccct acacagcact
901 tgcacagaag gcaatgtcaa gacatttcag gtgtttaaag gatgcaatag
951 gagcacaatt gaagcagagt tgtgagttat taggagagaa agatgcagga
1001 aattcgggat tgactaaagg agaaactccg aggtctaaga tgcttgaaca
1051 aagtttgagg caacaaaggg cgtttcacca aatgggaatg atggaacaag
1101 aagcttgagg accacaaaga ggcttacctg aacgttctgt caacatttta
20 1151 agagcttggc tttttgagca ttttctacac ccgtatccaa gtgatgctga
1201 taaacatctg ttggcaagac agactgggtc ctccagaaat caggtatcaa
1251 attggttcat taatgctagg gttcggttgt ggaaacccat ggtagaagat
1301 atgtatcaac aagaagccaa agatgaagat ggagatggag atgagaagag
1351 ccaaagccaa aacagtggca ataacataat tgcacaaaca ccaacgccta
25 1401 atagcctgac taacacttca tctactaata tgacgacgac aacagcccct
1451 acaactacga cagctctagc tgctgcagag acaggaacag ctgccactcc
1501 cataactggt acctcaagca aaagatccca aatcaatgcc acggatagtg
1551 acccttctact ttagcaatc aattccttct ctgaaaacca agctactttt
1601 ccgaccaaca ttcattgatc cgacgattgc cgtcgcggca acttatccgg
30 1651 tgacgacggg accaccacac atgatcatat ggggtccacc atgataaggt
1701 ttgggaccac tgctggtgac gtgtcactca ccttagggtt acgacatgca
1751 ggaaatttac cagagaatac tcatttcttt ggttaattaa tacgtatttt
1801 ccccatagta attaatataa actgaatttg cttgagctca tcataattta
1851 tgcattgctt tttgttataa gaaattccat aaattagctt tgtgttaaaa
35 1901 aaaaaaaaaa aaaaaaaaaa

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[0060] The nucleic acid sequence corresponding to SEQ ID NO:5 encodes a BEL transcription factor isolated from *Solanum tuberosum* identified herein as StBEL-13, which has a deduced amino acid sequence corresponding to SEQ ID NO:6 as follows:

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Met Val Met Gly Gly Gly Ala Ser Ser Gln Gln Leu Gly Tyr Ala Lys
1 5 10 15
45 Asn His Thr Pro Asn Val Ala Glu Ser Met Gln Leu Phe Leu Met Asn
20 25 30
Pro Gln Pro Arg Ser Pro Ser Pro Ser Pro Pro Asn Ser Thr Ser Ser
35 40 45
50 Thr Leu His Met Leu Leu Pro Asn Pro Ser Ser Thr Ser Thr Leu Gln
50 55 60
55 Gly Phe Pro Asn Pro Ala Glu Gly Ser Phe Gly Gln Phe Ile Thr Trp
65 70 75 80

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	Gly	Asn	Gly	Gly	Ala	Ser	Ala	Ala	Thr	Ala	Thr	His	His	Leu	Asn	Ala	
					85					90					95		
5	Gln	Asn	Glu	Ile	Gly	Gly	Val	Asn	Val	Val	Glu	Ser	Gln	Gly	Leu	Ser	
				100				105						110			
	Leu	Ser	Leu	Ser	Ser	Ser	Leu	Gln	His	Lys	Ala	Glu	Glu	Leu	Gln	Met	
			115					120					125				
10	Ser	Gly	Glu	Ala	Gly	Gly	Met	Met	Phe	Phe	Asn	Gln	Gly	Gly	Ser	Ser	
		130					135					140					
	Thr	Ser	Gly	Gln	Tyr	Arg	Tyr	Lys	Asn	Leu	Asn	Met	Gly	Gly	Ser	Gly	
	145					150					155					160	
15	Val	Ser	Pro	Asn	Ile	His	Gln	Val	His	Val	Gly	Tyr	Gly	Ser	Ser	Leu	
					165					170					175		
	Gly	Val	Val	Asn	Val	Leu	Arg	Asn	Ser	Lys	Tyr	Ala	Lys	Ala	Ala	Gln	
20				180					185					190			
	Glu	Leu	Leu	Glu	Glu	Phe	Cys	Ser	Val	Gly	Arg	Gly	Lys	Leu	Lys	Lys	
			195					200					205				
25	Thr	Asn	Asn	Lys	Ala	Ala	Ala	Asn	Asn	Pro	Asn	Thr	Asn	Pro	Ser	Gly	
		210					215					220					
	Ala	Asn	Asn	Glu	Ala	Ser	Ser	Lys	Asp	Val	Pro	Thr	Leu	Ser	Ala	Ala	
	225					230					235					240	
30	Asp	Arg	Ile	Glu	His	Gln	Arg	Arg	Lys	Val	Lys	Leu	Leu	Ser	Met	Val	
					245					250					255		
	Asp	Glu	Val	Asp	Arg	Arg	Tyr	Asn	His	Tyr	Cys	Glu	Gln	Met	Gln	Met	
35				260					265					270			
	Val	Val	Asn	Ser	Phe	Asp	Leu	Val	Met	Gly	Phe	Gly	Thr	Ala	Val	Pro	
			275					280					285				
40	Tyr	Thr	Ala	Leu	Ala	Gln	Lys	Ala	Met	Ser	Arg	His	Phe	Arg	Cys	Leu	
		290					295					300					
	Lys	Asp	Ala	Ile	Gly	Ala	Gln	Leu	Lys	Gln	Ser	Cys	Glu	Leu	Leu	Gly	
	305					310					315					320	
45	Glu	Lys	Asp	Ala	Gly	Asn	Ser	Gly	Leu	Thr	Lys	Gly	Glu	Thr	Pro	Arg	
					325					330					335		
	Leu	Lys	Met	Leu	Glu	Gln	Ser	Leu	Arg	Gln	Gln	Arg	Ala	Phe	His	Gln	
50				340					345					350			
	Met	Gly	Met	Met	Glu	Gln	Glu	Ala	Trp	Arg	Pro	Gln	Arg	Gly	Leu	Pro	
			355					360					365				
55	Glu	Arg	Ser	Val	Asn	Ile	Leu	Arg	Ala	Trp	Leu	Phe	Glu	His	Phe	Leu	
		370					375					380					
	His	Pro	Tyr	Pro	Ser	Asp	Ala	Asp	Lys	His	Leu	Leu	Ala	Arg	Gln	Thr	
60						390					395					400	

	Gly	Leu	Ser	Arg	Asn	Gln	Val	Ser	Asn	Trp	Phe	Ile	Asn	Ala	Arg	Val	
					405					410					415		
5	Arg	Leu	Trp	Lys	Pro	Met	Val	Glu	Asp	Met	Tyr	Gln	Gln	Glu	Ala	Lys	
				420					425					430			
	Asp	Glu	Asp	Gly	Asp	Gly	Asp	Glu	Lys	Ser	Gln	Ser	Gln	Asn	Ser	Gly	
			435					440					445				
10	Asn	Asn	Ile	Ile	Ala	Gln	Thr	Pro	Thr	Pro	Asn	Ser	Leu	Thr	Asn	Thr	
		450					455					460					
	Ser	Ser	Thr	Asn	Met	Thr	Thr	Thr	Thr	Ala	Pro	Thr	Thr	Thr	Thr	Ala	
15		465				470				475						480	
	Leu	Ala	Ala	Ala	Glu	Thr	Gly	Thr	Ala	Ala	Thr	Pro	Ile	Thr	Val	Thr	
					485				490						495		
20	Ser	Ser	Lys	Arg	Ser	Gln	Ile	Asn	Ala	Thr	Asp	Ser	Asp	Pro	Ser	Leu	
				500					505					510			
	Val	Ala	Ile	Asn	Ser	Phe	Ser	Glu	Asn	Gln	Ala	Thr	Phe	Pro	Thr	Asn	
			515					520					525				
25	Ile	His	Asp	Pro	Asp	Asp	Cys	Arg	Arg	Gly	Asn	Leu	Ser	Gly	Asp	Asp	
		530					535					540					
	Gly	Thr	Thr	Thr	His	Asp	His	Met	Gly	Ser	Thr	Met	Ile	Arg	Phe	Gly	
30		545				550					555					560	
	Thr	Thr	Ala	Gly	Asp	Val	Ser	Leu	Thr	Leu	Gly	Leu	Arg	His	Ala	Gly	
					565					570					575		
35	Asn	Leu	Pro	Glu	Asn	Thr	His	Phe	Phe	Gly							
				580					585								

The BEL transcription factor has a molecular mass of approximately 64.5 kDa. *StBEL-13*, isolated from *Solanum tuberosum*, has a single open reading frame ("ORF") of 1759 bp, extending between nucleotides 26-1784.

- 40 [0061] In a fourth embodiment, the BEL transcription factor from *Solanum tuberosum* is identified herein as StBEL-14 and is encoded by a nucleic acid molecule having a nucleotide sequence of SEQ ID NO:7 as follows:

45	1	aaccnaaaaa	agagatcgaa	ttcggcacga	gtgatcatgg	tccttcgtct	
	51	tctaagaaca	ttattagtga	acaattttac	caacatggta	gtcatgaaaa	
	101	tatggtgaca	acaacaacta	ctcatcatga	tgatcatcaa	ggctcgtggc	
	151	atcacgataa	taacagaaca	ttacttggtg	atgatccatc	tatgagatgt	
	201	gttttccctt	gtgaaggaaa	tgaaaggcca	agtcattgac	tttcattatc	
	251	tctttgttcc	tcaaattccat	caagtattgg	tttacaatct	tttgaactta	
50	301	gacatcaaga	tttgcaacaa	ggattaatac	atgatggatt	tttgggtaaa	
	351	tctacaaata	tacaacaagg	gtattttcat	catcatcatc	aagttaggga	
	401	ctcgaaatat	ttaggtccgg	ctcaagagtt	gctcagtgag	ttctgtagtc	

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451 tcggaataaa gaagaataat gatcattctt cttcaaaagt acttctaag
501 caacatgaga gtactgctag tacttcaaaa aagcaacttt tacagtctct
551 tgaccttttg gaacttcaaa aaagaaagac aaaattgctt caaatgcttg
601 aagagggtgga tagaaggtag aagcattatt gtgatcaaat gaaggctgtt
5   651 gtatcatcat ttgaagcagt ggctggaaat ggagcagcaa cagtttactc
701 agccttagca tcaagggcta tgtcaaggca ttttagatgt ttaagagatg
751 gaattgtggc acaaattaag gccacaaaaa tggctatggg agaaaaagac
801 agtactagta ctcttattcc tgggtcaaca agaggtgaaa caccaagact
851 cagacttctt gatcaaaactt taaggcaaca aaaggctttc caacagatga
10  901 atatgatgga gactcatcca tggagaccgc aacgtggtct cccagaaaga
951 tcagtctccg ttctccgcgc ttggctcttt gaacactttc ttcacccgta
1001 cccaagtgat gttgataaac acatttttagc tcgccaaact ggtctttcaa
1051 gaagccaggt gtctaattgg ttcattaatg caagggttaag gctatggaag
1101 ccaatggtgg aagaaatgta cttagaagaa acaaaagaag aagaaaatgt
15  1151 tggatctcca gatggatcaa aagccctaata tgatgacatg acaattcatc
1201 aatcacacat tgatcatcat caagctgac aaaagccaaa tcttgaaga
1251 attgactctg aatgcataatc ttccatcata aatcatcaac ctcatgagaa
1301 aaatgatcaa aactatggag taattagagg tggagatcaa tcgtttggcg
1351 cgattgagct agatttttca acaaatattg cttatggtac tagtggtggt
20  1401 gaccatcatc atcatggagg ggggtgttct ttaacattgg gattacaaca
1451 acatggtgga agtggtggat catcaatggg gtttaactaca ttttcatcac
1501 aaccatctca taatcaaagt tcactttttt atccaagaga tgaatgacaa
1551 gttcaatatt catcactttt ggatagtga aatcagaatt tgccatatag
1601 aaaccttgat gggggcacia cttcttcatg atttggtctg ttaaaaaatg
25  1651 acagagattc ttcatttttg accttattat atactctaata ttaatatat
1701 attggtgatg aatgatgata aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa
1751 aaaaaaaaaa acctcgancc cggctcgactn tanancccta tagngagtcg
1801 tntnctgca nanatctntg aatcgtaaat nctgaaaaac cccgcaagtt
1851 cacttcaact gngcatcgng cnccatctca atttctttca tttatnctc
30  1901 gttttgcctt ntttatgta actatnctcc tntaagtttc aatcttgccc
1951 atgtaacctn tgatctntaa aattttttta atgactanaa ttaatgccca
2001 tntttttttt ggacctaaat tnttcatgaa aatntnttnc nagggttnt
2051 tcaaaaanctt tggacttntt cnccanaggt ttggtcaagt ntccaatcaa
2101 ggt

```

[0062] The nucleic acid sequence corresponding to SEQ ID NO:7 encodes a BEL transcription factor isolated from *Solanum tuberosum* identified herein as StBEL-14, which has a deduced amino acid sequence corresponding to SEQ ID NO:8 as follows:

```

40 Met Val Asn His Gln Leu Gln Asn Phe Glu Thr Asn Pro Glu Met Tyr
    1             5             10             15
Asn Leu Ser Ser Thr Thr Ser Ser Met Asp Gln Met Ile Gly Phe Pro
45             20             25             30
Pro Asn Asn Asn Asn Pro His His Val Leu Trp Lys Gly Asn Phe Pro
    35             40             45
50 Asn Lys Ile Asn Gly Val Asp Asp Asp Asp His Gly Pro Ser Ser Ser
    50             55             60
Lys Asn Ile Ile Ser Glu Gln Phe Tyr Gln His Gly Ser His Glu Asn
55    65             70             75             80
Met Leu Thr Thr Thr Thr Thr His His Asp Asp His Gln Gly Ser Trp
    85             90             95

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	His	His	Asp	Asn	Asn	Arg	Thr	Leu	Leu	Val	Asp	Asp	Pro	Ser	Met	Arg	
				100					105					110			
5	Cys	Val	Phe	Pro	Cys	Glu	Gly	Asn	Glu	Arg	Pro	Ser	His	Gly	Leu	Ser	
			115					120					125				
	Leu	Ser	Leu	Cys	Ser	Ser	Asn	Pro	Ser	Ser	Ile	Gly	Leu	Gln	Ser	Phe	
		130					135					140					
10	Glu	Leu	Arg	His	Gln	Asp	Leu	Gln	Gln	Gly	Leu	Ile	His	Asp	Gly	Phe	
	145					150					155					160	
	Leu	Gly	Lys	Ser	Thr	Asn	Ile	Gln	Gln	Gly	Tyr	Phe	His	His	His	His	
15					165					170					175		
	Gln	Val	Arg	Asp	Ser	Lys	Tyr	Leu	Gly	Pro	Ala	Gln	Glu	Leu	Leu	Ser	
				180					185					190			
20	Glu	Phe	Cys	Ser	Leu	Gly	Ile	Lys	Lys	Asn	Asn	Asp	His	Ser	Ser	Ser	
		195						200					205				
	Lys	Val	Leu	Leu	Lys	Gln	His	Glu	Ser	Thr	Ala	Ser	Thr	Ser	Lys	Lys	
		210					215					220					
25	Gln	Leu	Leu	Gln	Ser	Leu	Asp	Leu	Leu	Glu	Leu	Gln	Lys	Arg	Lys	Thr	
	225					230					235					240	
	Lys	Leu	Leu	Gln	Met	Leu	Glu	Glu	Val	Asp	Arg	Arg	Tyr	Lys	His	Tyr	
30					245					250					255		
	Cys	Asp	Gln	Met	Lys	Ala	Val	Val	Ser	Ser	Phe	Glu	Ala	Val	Ala	Gly	
				260					265					270			
35	Asn	Gly	Ala	Ala	Thr	Val	Tyr	Ser	Ala	Leu	Ala	Ser	Arg	Ala	Met	Ser	
		275						280					285				
	Arg	His	Phe	Arg	Cys	Leu	Arg	Asp	Gly	Ile	Val	Ala	Gln	Ile	Lys	Ala	
		290					295					300					
40	Thr	Lys	Met	Ala	Met	Gly	Glu	Lys	Asp	Ser	Thr	Ser	Thr	Leu	Ile	Pro	
	305					310					315					320	
	Gly	Ser	Thr	Arg	Gly	Glu	Thr	Pro	Arg	Leu	Arg	Leu	Leu	Asp	Gln	Thr	
45					325					330					335		
	Leu	Arg	Gln	Gln	Lys	Ala	Phe	Gln	Gln	Met	Asn	Met	Met	Glu	Thr	His	
				340					345					350			
50	Pro	Trp	Arg	Pro	Gln	Arg	Gly	Leu	Pro	Glu	Arg	Ser	Val	Ser	Val	Leu	
		355						360					365				
	Arg	Ala	Trp	Leu	Phe	Glu	His	Phe	Leu	His	Pro	Tyr	Pro	Ser	Asp	Val	
		370					375					380					
55	Asp	Lys	His	Ile	Leu	Ala	Arg	Gln	Thr	Gly	Leu	Ser	Arg	Ser	Gln	Val	
	385					390					395					400	
	Ser	Asn	Trp	Phe	Ile	Asn	Ala	Arg	Val	Arg	Leu	Trp	Lys	Pro	Met	Val	
60					405					410					415		

	Glu	Glu	Met	Tyr	Leu	Glu	Glu	Thr	Lys	Glu	Glu	Glu	Asn	Val	Gly	Ser	
				420					425					430			
5	Pro	Asp	Gly	Ser	Lys	Ala	Leu	Ile	Asp	Asp	Met	Thr	Ile	His	Gln	Ser	
			435					440					445				
	His	Ile	Asp	His	His	Gln	Ala	Asp	Gln	Lys	Pro	Asn	Leu	Val	Arg	Ile	
		450					455					460					
10	Asp	Ser	Glu	Cys	Ile	Ser	Ser	Ile	Ile	Asn	His	Gln	Pro	His	Glu	Lys	
	465					470				475						480	
	Asn	Asp	Gln	Asn	Tyr	Gly	Val	Ile	Arg	Gly	Gly	Asp	Gln	Ser	Phe	Gly	
15					485					490					495		
	Ala	Ile	Glu	Leu	Asp	Phe	Ser	Thr	Asn	Ile	Ala	Tyr	Gly	Thr	Ser	Gly	
				500					505					510			
20	Gly	Asp	His	His	His	His	Gly	Gly	Gly	Val	Ser	Leu	Thr	Leu	Gly	Leu	
			515					520					525				
	Gln	Gln	His	Gly	Gly	Ser	Gly	Gly	Ser	Ser	Met	Gly	Leu	Thr	Thr	Phe	
		530					535					540					
25	Ser	Ser	Gln	Pro	Ser	His	Asn	Gln	Ser	Ser	Leu	Phe	Tyr	Pro	Arg	Asp	
	545					550					555					560	
	Asp	Asp	Gln	Val	Gln	Tyr	Ser	Ser	Leu	Leu	Asp	Ser	Glu	Asn	Gln	Asn	
30					565					570					575		
	Leu	Pro	Tyr	Arg	Asn	Leu	Asp	Gly	Gly	Thr	Thr	Ser	Ser				
				580					585								

The BEL transcription factor has a molecular mass of approximately 64.8 kDa.

35 *StBEL-14*, isolated from *Solanum tuberosum*, has a single open reading frame ("ORF") of 1768 bp, extending between nucleotides 85-1852.

[0063] In a fifth embodiment, the BEL transcription factor from *Solanum tuberosum* is identified herein as StBEL-22 and is encoded by a nucleic acid molecule having a nucleotide sequence of SEQ ID NO:9 as follows:

40	1	acgagcg	ttt	atgagac	agc	cgggttg	ttg	tctgaa	atgt	tcaatt	tttca	gacaac	atcc	
	61	acggctg	caa	ctgaatt	gtt	gcagaat	caa	ttgtcaa	aata	actatag	aca	cccga	atcaa	
	121	cagccac	atc	atcaact	ccc	gaccagg	gag	tggtttg	gta	acagaca	aga	gatcg	tagtt	
45	181	ggtgga	agt	tgcaggt	aac	atttg	gggat	acaaaag	atg	atgtga	atgc	gaaggt	atata	
	241	ttgagta	acc	gtgatag	tgt	aactgat	tatt	tatcagc	gtc	aacaca	aatca	agtacca	aagt	
	301	ataaat	accg	cggagt	ccat	gcaact	tttt	cttatga	atc	cacaacca	aag	ttcacc	atca	
	361	caatcta	ctc	cttcaact	ctc	tcataca	agg	ttttcta	g	cggtcg	gag	gcatttt	tagt	
	421	caattca	tgt	gtggagg	agc	aagtact	tct	tcaaat	ccaa	ttggagg	agt	aaatgt	gatt	
50	481	gatcaag	ggc	aaggtct	ttc	attgtcc	ttg	tcatact	actt	tacaaca	attt	ggaagc	atcc	
	541	aaagtgg	aaag	atttgagg	at	gaatagt	gga	ggagaa	atgt	tgttttt	caa	tcaaga	aaagt	
	601	caaaat	catc	ataatatt	gg	ttttggg	tca	tcactag	gac	tagtcaa	tgt	gttgagg	aat	
	661	tcaaagt	atg	tcaaagc	aac	acaagag	ttg	ttggaag	agt	ttgtgt	gtg	tgggaag	ggg	
	721	caattgt	tca	agaaaat	caa	caaagtt	tct	aggaata	aaca	acacaag	tac	atcaccc	att	
55	781	attaacc	cta	gtggaag	taa	taacaata	aat	tcattct	tct	caaagg	ctat	tatocct	cct	
	841	aatttgt	caa	ctgcagag	ag	acttgat	cat	caaaga	agga	aggtcaa	aact	tttatcc	atg	
	901	cttgatg	agg	tagagaaa	ag	atacaacc	ac	tattgtg	aac	aaatgcag	at	ggtagtaa	ac	

```

5  961 tcattcgatc tagtgatggg ttttggagct gcagttcctt acacagcact agcacagaaa
1021 gccatgtcta ggcattttcaa gtgttttaaaa gatggcggtg cggcgcaatt gaagaagaca
1081 tgtgaggcac taggtgaaaa agatgcaagc agtagttcag gactgactaa aggagaaaca
1141 ccaaggctta aggtgcttga acaaagcttg aggcaacaaa gagcttttca acaaattgga
1201 atgatggaac aagaagcttg gaggccacaa agaggattgc ctgaacgac tgccaatatt
1261 ttaagagctt ggcttttcga acattttcta catccgtatc caagtgatgc agataagcat
1321 cttttggcac gacagactgg tctctccaga aaccaggtag caaactggtt cataaatgcg
1381 aggggtgagat tgtggaaacc catggtagaa gaaatgtatc aaagagaggt taatgaagat
1441 gatgttgatg acatgcaaga aaacacaaac agtacaaata cacaaatacc aacgcctaatt
1501 attattatta caaccaattc taacattaca gaaacaaaat cagctgccac tgccacaatt
1561 gcttcagaca aaaaacccca aatcaatgtc tctgaaattg acccttcaat tgcgcaatg
1621 aatacacatt attcttcctc tatgccaaact caattaacca atttcccac tattcaagat
1681 gagtccgacc acatcttata tcgccgcagt ggagcgggaat atggggaccac aaatatggct
1741 agtaattctg aaattggatc caacatgata acatttggga ccactacggc tagtgatggt
1801 tcacttacct taggactgcg ccatgcggtt aatttacctg agaatactca tttttccggt
1861 taattaagat agtgtattca aacactgcta cataaattat gatatttatat atatatatat
1921 tgcacccga ttagtttat

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[0064] The nucleic acid sequence corresponding to SEQ ID NO:9 encodes  
 20 a BEL transcription factor isolated from *Solanum tuberosum* identified herein as  
 StBEL-22, which has a deduced amino acid sequence corresponding to SEQ ID  
 NO:10 as follows:

```

25  Thr Ser Val Tyr Glu Thr Ala Gly Leu Leu Ser Glu Met Phe Asn Phe
    1          5          10          15
    Gln Thr Thr Ser Thr Ala Ala Thr Glu Leu Leu Gln Asn Gln Leu Ser
        20          25          30
30  Asn Asn Tyr Arg His Pro Asn Gln Gln Pro His His Gln Pro Pro Thr
    35          40          45
    Arg Glu Trp Phe Gly Asn Arg Gln Glu Ile Val Val Gly Gly Ser Leu
    50          55          60
35  Gln Val Thr Phe Gly Asp Thr Lys Asp Asp Val Asn Ala Lys Val Leu
    65          70          75          80
    Leu Ser Asn Arg Asp Ser Val Thr Asp Tyr Tyr Gln Arg Gln His Asn
    85          90          95
    Gln Val Pro Ser Ile Asn Thr Ala Glu Ser Met Gln Leu Phe Leu Met
        100          105          110
45  Asn Pro Gln Pro Ser Ser Pro Ser Gln Ser Thr Pro Ser Thr Leu His
    115          120          125
    Gln Gly Phe Ser Ser Pro Val Gly Gly His Phe Ser Gln Phe Met Cys
    130          135          140
50  Gly Gly Ala Ser Thr Ser Ser Asn Pro Ile Gly Gly Val Asn Val Ile
    145          150          155          160
    Asp Gln Gly Gln Gly Leu Ser Leu Ser Leu Ser Ser Thr Leu Gln His
    165          170          175
55  Leu Glu Ala Ser Lys Val Glu Asp Leu Arg Met Asn Ser Gly Gly Glu
    180          185          190

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	Met	Leu	Phe	Phe	Asn	Gln	Glu	Ser	Gln	Asn	His	His	Asn	Ile	Gly	Phe	
			195					200					205				
5	Gly	Ser	Ser	Leu	Gly	Leu	Val	Asn	Val	Leu	Arg	Asn	Ser	Lys	Tyr	Val	
		210					215					220					
	Lys	Ala	Thr	Gln	Glu	Leu	Leu	Glu	Glu	Phe	Cys	Cys	Val	Gly	Lys	Gly	
10		225				230					235					240	
	Gln	Leu	Phe	Lys	Lys	Ile	Asn	Lys	Val	Ser	Arg	Asn	Asn	Asn	Thr	Ser	
				245						250					255		
15	Thr	Ser	Pro	Ile	Ile	Asn	Pro	Ser	Gly	Ser	Asn	Asn	Asn	Asn	Ser	Ser	
			260						265					270			
	Ser	Ser	Lys	Ala	Ile	Ile	Pro	Pro	Asn	Leu	Ser	Thr	Ala	Glu	Arg	Leu	
			275					280					285				
20	Asp	His	Gln	Arg	Arg	Lys	Val	Lys	Leu	Leu	Ser	Met	Leu	Asp	Glu	Val	
		290					295					300					
	Glu	Lys	Arg	Tyr	Asn	His	Tyr	Cys	Glu	Gln	Met	Gln	Met	Val	Val	Asn	
25		305				310					315					320	
	Ser	Phe	Asp	Leu	Val	Met	Gly	Phe	Gly	Ala	Ala	Val	Pro	Tyr	Thr	Ala	
				325						330					335		
30	Leu	Ala	Gln	Lys	Ala	Met	Ser	Arg	His	Phe	Lys	Cys	Leu	Lys	Asp	Gly	
			340						345					350			
	Val	Ala	Ala	Gln	Leu	Lys	Lys	Thr	Cys	Glu	Ala	Leu	Gly	Glu	Lys	Asp	
			355					360					365				
35	Ala	Ser	Ser	Ser	Ser	Gly	Leu	Thr	Lys	Gly	Glu	Thr	Pro	Arg	Leu	Lys	
		370					375					380					
	Val	Leu	Glu	Gln	Ser	Leu	Arg	Gln	Gln	Arg	Ala	Phe	Gln	Gln	Met	Gly	
40		385				390					395					400	
	Met	Met	Glu	Gln	Glu	Ala	Trp	Arg	Pro	Gln	Arg	Gly	Leu	Pro	Glu	Arg	
					405					410					415		
45	Ser	Val	Asn	Ile	Leu	Arg	Ala	Trp	Leu	Phe	Glu	His	Phe	Leu	His	Pro	
			420						425					430			
	Tyr	Pro	Ser	Asp	Ala	Asp	Lys	His	Leu	Leu	Ala	Arg	Gln	Thr	Gly	Leu	
			435					440					445				
50	Ser	Arg	Asn	Gln	Val	Ala	Asn	Trp	Phe	Ile	Asn	Ala	Arg	Val	Arg	Leu	
		450					455					460					
	Trp	Lys	Pro	Met	Val	Glu	Glu	Met	Tyr	Gln	Arg	Glu	Val	Asn	Glu	Asp	
55		465				470					475					480	
	Asp	Val	Asp	Asp	Met	Gln	Glu	Asn	Gln	Asn	Ser	Thr	Asn	Thr	Gln	Ile	
					485					490					495		
60	Pro	Thr	Pro	Asn	Ile	Ile	Ile	Thr	Thr	Asn	Ser	Asn	Ile	Thr	Glu	Thr	
				500					505					510			



	Lys	Ser	Ala	Ala	Thr	Ala	Thr	Ile	Ala	Ser	Asp	Lys	Lys	Pro	Gln	Ile
			515					520					525			
5	Asn	Val	Ser	Glu	Ile	Asp	Pro	Ser	Ile	Val	Ala	Met	Asn	Thr	His	Tyr
	530						535					540				
	Ser	Ser	Ser	Met	Pro	Thr	Gln	Leu	Thr	Asn	Phe	Pro	Thr	Ile	Gln	Asp
10	545					550					555					560
	Glu	Ser	Asp	His	Ile	Leu	Tyr	Arg	Arg	Ser	Gly	Ala	Glu	Tyr	Gly	Thr
					565					570					575	
	Thr	Asn	Met	Ala	Ser	Asn	Ser	Glu	Ile	Gly	Ser	Asn	Met	Ile	Thr	Phe
15				580					585					590		
	Gly	Thr	Thr	Thr	Ala	Ser	Asp	Val	Ser	Leu	Thr	Leu	Gly	Leu	Arg	His
				595				600					605			
20	Ala	Gly	Asn	Leu	Pro	Glu	Asn	Thr	His	Phe	Ser	Gly				
	610						615					620				

The BEL transcription factor has a molecular mass of approximately 67.3 kDa.

*StBEL-22*, isolated from *Solanum tuberosum*, has a single open reading frame

25 ("ORF") of 1863 bp, extending between nucleotides 1-1863.

[0065] In a sixth embodiment, the BEL transcription factor from *Solanum tuberosum* is identified herein as *StBEL-29* and is encoded by a nucleic acid molecule having a nucleotide sequence of SEQ ID NO:11 as follows:

30	1	caagggtctt	cacttagcct	gtcctcgtcc	cagcagccgg	ggtttgggaa	cttcacggcg
	61	gcgcgtgagc	ttgtttcttc	gccttcgggt	tcggcttcag	cttcagggat	acaacaacaa
	121	caacagcaac	aacagagtat	tagtagtggt	cctttgagtt	ctaagtacat	gaaggctgca
	181	caagagctac	ttgatgaagt	tgtaaatggt	ggaaaatcaa	tgaaaagtac	taatagtact
35	241	gatgttgttg	ttaataatga	tgtcaagaaa	tcgaagaata	tggtcgatat	ggacggacag
	301	ttagacggag	ttggagcaga	caaagacgga	gctccaacaa	ctgagctaag	tacaggggag
	361	agacaagaaa	ttcaaatgaa	gaaagcaaaa	cttgtaaca	tgcttgacga	ggtggagcag
	421	aggtatagac	attatcatca	ccaaatgcag	tcagtgtata	attggttaga	gcaagctgct
	481	ggcattggat	cagcaaaaac	atatacagca	ttggctttgc	agacgatttc	gaagcaattt
	541	aggtgtctta	aggacgcgat	aattgggtcaa	atacgtatcag	caagccagac	gttagggcaa
40	601	gaagatagtt	tgggagggaa	gattgaaggt	tcaaggctta	aatttggtga	taatcagcta
	661	agacagcaaa	gggctttgca	acaattggga	atgatccagc	ataatgcttg	gagacctcag
	721	agaggattgc	ccgaacgagc	tgtttctggt	cttcgcgctt	ggctttttga	acatttcctc
	781	catccttata	ccaaggattc	agacaaaatg	atgctagcaa	aacaacagg	actaactagg
45	841	agtcagggtg	cgaattgggt	catcaatgct	cgagttcgct	tttgaagcc	aatggtggaa
	901	gagatgtact	tgggaagagat	aaaagaacac	gaacagaatg	ggttgggtca	agaaaagacg
	961	agcaaattag	gtgaacagaa	cgaagattca	acaacatcaa	gatccattgc	tacacaagac
	1021	aaaagccctg	gttcagatag	ccaaaacaag	agttttgtct	caaaacagga	caatcatttg
	1081	cctcaacaca	accctgcttc	accaaagccc	gatgtccaac	gccacttcca	tacccttata
	1141	ggtagaccca	tccgtaatca	gtctgctggt	ttcaacctca	ttggatcacc	agagatcgaa
50	1201	agcatcaaca	ttactcaagg	gagtcctaaa	aaaccgagga	acaacgagat	gttgcatcca
	1261	ccaacacagca	ttccatccat	caacatggat	gtaaagccta	acgaggaaca	aatgtcagtg
	1321	aagtttgggt	atgataggca	ggacagagat	ggattctcac	taatgggagg	accgatgaac
	1381	ttcatgggag	gattcggagc	ctatccatt	ggagaaattg	ctcggtttag	caccgagcaa
	1441	ttctcagcac	catactcaac	cagtggtcaca	gtttcactca	ctcttggcct	accacataac
55	1501	gaaaacctct	caatgtctgc	aacacaccac	agtttccttc	caattccaac	acaaaacatc
	1561	caaattggaa	gtgaacaaaa	tcagtgtgtt	ggtagcttaa	acacaccaac	atcagctcac
	1621	tcaacatcaa	gcgtctatga	aaccttcaac	attcagaaca	gaaagaggtt	cgccgcaccc
	1681	ttgttaccag	attttgttgc	ctgatcaca	aaacaaaaac	aggttttggc	aacagacaaa
	1741	cttctgtcgc	taaacaagga	catgatttag	cgacagataa	cttcagtcgc	taacttagcg

1801 actgaaaact tctgtcgcta agcatgaaca tgtattagcg acatacagta tgcaactgta  
 1861 tgtcactaaa caagaacatg atgaattagt gacggacaac ttctgtcgct aaacaacaaa  
 1921 aaaaaatcca tgtttttagta tattgtttct cattctatca tatcatggta gtgtaaagaa  
 1981 tcaagaaaca agttttacat agtaacagtc ttatacatt ggagatgaag aaccatttaa  
 2041 gttcttcaaa atagatagat tttctagggt acttctanaa gatatatata tggttgaggg  
 2101 tttgtatatt aaaaaaaaaa aaaaaaaa

[0066] The nucleic acid sequence corresponding to SEQ ID NO:11  
 encodes a BEL transcription factor isolated from *Solanum tuberosum* identified  
 herein as StBEL-29, which has a deduced amino acid sequence corresponding to  
 SEQ ID NO:12 as follows:

15	Gln Gly Leu Ser Leu Ser Leu Ser Ser Ser Gln Gln Pro Gly Phe Gly	1 5 10 15
	Asn Phe Thr Ala Ala Arg Glu Leu Val Ser Ser Pro Ser Gly Ser Ala	20 25 30
20	Ser Ala Ser Gly Ile Gln Gln Gln Gln Gln Gln Gln Ser Ile Ser	35 40 45
	Ser Val Pro Leu Ser Ser Lys Tyr Met Lys Ala Ala Gln Glu Leu Leu	50 55 60
25	Asp Glu Val Val Asn Val Gly Lys Ser Met Lys Ser Thr Asn Ser Thr	65 70 75 80
	Asp Val Val Val Asn Asn Asp Val Lys Lys Ser Lys Asn Met Gly Asp	85 90 95
30	Met Asp Gly Gln Leu Asp Gly Val Gly Ala Asp Lys Asp Gly Ala Pro	100 105 110
35	Thr Thr Glu Leu Ser Thr Gly Glu Arg Gln Glu Ile Gln Met Lys Lys	115 120 125
	Ala Lys Leu Val Asn Met Leu Asp Glu Val Glu Gln Arg Tyr Arg His	130 135 140
40	Tyr His His Gln Met Gln Ser Val Ile His Trp Leu Glu Gln Ala Ala	145 150 155 160
	Gly Ile Gly Ser Ala Lys Thr Tyr Thr Ala Leu Ala Leu Gln Thr Ile	165 170 175
45	Ser Lys Gln Phe Arg Cys Leu Lys Asp Ala Ile Ile Gly Gln Ile Arg	180 185 190
50	Ser Ala Ser Gln Thr Leu Gly Glu Glu Asp Ser Leu Gly Gly Lys Ile	195 200 205
	Glu Gly Ser Arg Leu Lys Phe Val Asp Asn Gln Leu Arg Gln Gln Arg	210 215 220
55	Ala Leu Gln Gln Leu Gly Met Ile Gln His Asn Ala Trp Arg Pro Gln	

	225		230		235		240
	Arg Gly Leu Pro	Glu Arg Ala Val Ser Val Leu Arg Ala Trp Leu Phe					
		245		250			255
5	Glu His Phe Leu	His Pro Tyr Pro Lys Asp Ser Asp Lys Met Met Leu					
		260		265			270
10	Ala Lys Gln Thr	Gly Leu Thr Arg Ser Gln Val Ser Asn Trp Phe Ile					
		275		280			285
	Asn Ala Arg Val	Arg Leu Trp Lys Pro Met Val Glu Glu Met Tyr Leu					
		290		295			300
15	Glu Glu Ile Lys	Glu His Glu Gln Asn Gly Leu Gly Gln Glu Lys Thr					
		305		310			315
	Ser Lys Leu Gly	Glu Gln Asn Glu Asp Ser Thr Thr Ser Arg Ser Ile					
		325		330			335
20	Ala Thr Gln Asp	Lys Ser Pro Gly Ser Asp Ser Gln Asn Lys Ser Phe					
		340		345			350
	Val Ser Lys Gln	Asp Asn His Leu Pro Gln His Asn Pro Ala Ser Pro					
		355		360			365
25	Met Pro Asp Val	Gln Arg His Phe His Thr Pro Ile Gly Met Thr Ile					
		370		375			380
30	Arg Asn Gln Ser	Ala Gly Phe Asn Leu Ile Gly Ser Pro Glu Ile Glu					
		385		390			395
	Ser Ile Asn Ile	Thr Gln Gly Ser Pro Lys Lys Pro Arg Asn Asn Glu					
		405		410			415
35	Met Leu His Ser	Pro Asn Ser Ile Pro Ser Ile Asn Met Asp Val Lys					
		420		425			430
40	Pro Asn Glu Glu	Gln Met Ser Met Lys Phe Gly Asp Asp Arg Gln Asp					
		435		440			445
	Arg Asp Gly Phe	Ser Leu Met Gly Gly Pro Met Asn Phe Met Gly Gly					
		450		455			460
45	Phe Gly Ala Tyr	Pro Ile Gly Glu Ile Ala Arg Phe Ser Thr Glu Gln					
		465		470			475
	Phe Ser Ala Pro	Tyr Ser Thr Ser Gly Thr Val Ser Leu Thr Leu Gly					
		485		490			495
50	Leu Pro His Asn	Glu Asn Leu Ser Met Ser Ala Thr His His Ser Phe					
		500		505			510
55	Leu Pro Ile Pro	Thr Gln Asn Ile Gln Ile Gly Ser Glu Pro Asn His					
		515		520			525
	Glu Phe Gly Ser	Leu Asn Thr Pro Thr Ser Ala His Ser Thr Ser Ser					
		530		535			540
60	Val Tyr Glu Thr	Phe Asn Ile Gln Asn Arg Lys Arg Phe Ala Ala Pro					

545 550 555 560

Leu Leu Pro Asp Phe Val Ala  
565

5

The BEL transcription factor has a molecular mass of approximately 56.2 kDa. *StBEL-29*, isolated from *Solanum tuberosum*, has a single open reading frame ("ORF") of 1704 bp, extending between nucleotides 1-1704.

- 10 [0067] In a seventh embodiment, the BEL transcription factor from *Solanum tuberosum* is identified herein as StBEL-30 and is encoded by a nucleic acid molecule having a nucleotide sequence of SEQ ID NO:13 as follows:

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15      1 atctccaagt aaaaagggtta ttgagaaaag taacacagat ggcgacttat tttcctagtc
        61 caaacaatca aagagatgct gatcagacat ttcaatatatt taggcaatct ttgcctgagt
      121 cttattcaga agcttcaaat gctccagaaa acatgatggt attcatgaac tattcttctt
      181 ctggggcata ttcatgatatg ttgacgggta ctcccaaca acaacacaac tgcatacgata
      241 tcccatctat aggagccacg cctttcaaca catcccaaca agaaatattg tcaaatcttg
      301 gaggatcgca gatggggatt caggattttt ctcatggag agatagcaga aatgagatgc
      361 tagctgataa tgtctttcaa gttgcacaaa atgtgcaggg tcaaggatta tccctcagtc
      421 ttggctccaa tataccatct ggaattggaa ttccacatgt ccaatctcag aatcctaacc
      481 aagggtggcg ttttaacatg tcctttggag atggtgataa ttcccaacca aaagaacaaa
      541 gaaatgcaga ttattttcct ccggataatc ctggaaggga cttggatgct atgaaagggg
      601 ataatttccc atatggtacg tcgagatttg caaggaccat tcccagctcg aagtatttga
      661 aagcagctca atatttgctt gatgaggttg ttagtgctag aaaggccatc aaggagcaaa
      721 attctaagaa agagttgaca aaggattcca gagagtctga tgtggactcg aaaaatatat
      781 catcagatac tcctgcaaat ggggggtcaa atcctcatga gtccaaaaac aaccaaagtg
      841 aactttcacc taccgagaag caagaagtgc agaacaaact ggccaaactt ctgtcaatgc
      901 tggatgagat tgatagaagg tacagacaat attatcatca gatgcaaata gtggtttcat
      961 catttgatgt gtagctgga gaaggagcag cttaaaccata cacagctctt gctctccaga
     1021 caatttcccg acacttccgt tgcttgctg atgcaatctg cgatcagatt cgagcatcac
     1081 gaagaagtct tggagagcaa gatgcttcag aaaacagcaa agcgattgga atatcacgcc
     1141 tgcgttttgt ggatcatcat attagacagc agagagccct gcagcagctt ggtatgatgc
     1201 aacaacatgc ctggaggcct cagaggggat tgccctgaaag ctctgtttca gttttgcgtg
     1261 cttggctctt tgagcacttt cttcatccct acccgaaaga ttctgacaaa attatgctag
     1321 caaggcaaac tggcttaacg agaagtcagg tatcaaatgt gttcataaat gcacgggtgc
     1381 gtcttttgaa acccatggtt gaggaatgt acaaagaaga ggctggtgat gctaaaatag
     1441 actcaaattc ttcatcggat gttgccccca gacttgcaac aaaagactca aaagttgaag
     1501 aaagaggaga attgcaccag aatgcagctt cagaatttga gcagtacaat agtggccaaa
     1561 tcctggagtc aaaatctaac catgaagctg atgtagaat ggagggagca agtaatgcag
     1621 aaactcaaa gcaatctgga atggaaaacc aaacaggcga acccctgcct gctatggata
     1681 attgcaccct ttttcaggac gcatttggtc aaagcaacga tagattctca gaatttgta
     1741 gttttggaag tggaaatgta ctaccaatg gagtttact tacattggg ctgcagcaag
     1801 gtgaaggga caacctacct atgtccatcg aaactcacgt tagttatgta ccattaaggg
     1861 cagatgacat gtatagtaca gcacctacta ctatgggtccc tgaacacga gaattcaact
     1921 gcttggtatc tgggaatagg cagcaaccat tttggctcct accatctgct acatgatttt
     1981 gtatgtgttg tagaattaaa ctgcaagttt tgagtacatc aacattcatc ttcaaaaaaa
     2041 aaaaaaaaaa aaaaaaaaaa aaaaaa

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- 50 [0068] The nucleic acid sequence corresponding to SEQ ID NO:13 encodes a BEL transcription factor isolated from *Solanum tuberosum* identified herein as StBEL-30, which has a deduced amino acid sequence corresponding to SEQ ID NO:14 as follows:

	Met	Ala	Thr	Tyr	Phe	Pro	Ser	Pro	Asn	Asn	Gln	Arg	Asp	Ala	Asp	Gln	
	1				5					10					15		
5	Thr	Phe	Gln	Tyr	Phe	Arg	Gln	Ser	Leu	Pro	Glu	Ser	Tyr	Ser	Glu	Ala	
				20					25					30			
	Ser	Asn	Ala	Pro	Glu	Asn	Met	Met	Val	Phe	Met	Asn	Tyr	Ser	Ser	Ser	
			35					40					45				
10	Gly	Ala	Tyr	Ser	Asp	Met	Leu	Thr	Gly	Thr	Ser	Gln	Gln	Gln	His	Asn	
		50					55					60					
	Cys	Ile	Asp	Ile	Pro	Ser	Ile	Gly	Ala	Thr	Pro	Phe	Asn	Thr	Ser	Gln	
15	65					70					75					80	
	Gln	Glu	Ile	Leu	Ser	Asn	Leu	Gly	Gly	Ser	Gln	Met	Gly	Ile	Gln	Asp	
					85					90						95	
20	Phe	Ser	Ser	Trp	Arg	Asp	Ser	Arg	Asn	Glu	Met	Leu	Ala	Asp	Asn	Val	
				100					105					110			
	Phe	Gln	Val	Ala	Gln	Asn	Val	Gln	Gly	Gln	Gly	Leu	Ser	Leu	Ser	Leu	
25			115					120					125				
	Gly	Ser	Asn	Ile	Pro	Ser	Gly	Ile	Gly	Ile	Ser	His	Val	Gln	Ser	Gln	
		130					135					140					
	Asn	Pro	Asn	Gln	Gly	Gly	Gly	Phe	Asn	Met	Ser	Phe	Gly	Asp	Gly	Asp	
30	145					150					155					160	
	Asn	Ser	Gln	Pro	Lys	Glu	Gln	Arg	Asn	Ala	Asp	Tyr	Phe	Pro	Pro	Asp	
					165					170					175		
35	Asn	Pro	Gly	Arg	Asp	Leu	Asp	Ala	Met	Lys	Gly	Tyr	Asn	Ser	Pro	Tyr	
				180					185					190			
	Gly	Thr	Ser	Ser	Ile	Ala	Arg	Thr	Ile	Pro	Ser	Ser	Lys	Tyr	Leu	Lys	
40			195					200					205				
	Ala	Ala	Gln	Tyr	Leu	Leu	Asp	Glu	Val	Val	Ser	Val	Arg	Lys	Ala	Ile	
		210					215					220					
	Lys	Glu	Gln	Asn	Ser	Lys	Lys	Glu	Leu	Thr	Lys	Asp	Ser	Arg	Glu	Ser	
45	225					230					235					240	
	Asp	Val	Asp	Ser	Lys	Asn	Ile	Ser	Ser	Asp	Thr	Pro	Ala	Asn	Gly	Gly	
					245					250					255		
50	Ser	Asn	Pro	His	Glu	Ser	Lys	Asn	Asn	Gln	Ser	Glu	Leu	Ser	Pro	Thr	
				260					265					270			
	Glu	Lys	Gln	Glu	Val	Gln	Asn	Lys	Leu	Ala	Lys	Leu	Leu	Ser	Met	Leu	
		275						280					285				
55	Asp	Glu	Ile	Asp	Arg	Arg	Tyr	Arg	Gln	Tyr	Tyr	His	Gln	Met	Gln	Ile	
		290					295					300					

	Val	Val	Ser	Ser	Phe	Asp	Val	Val	Ala	Gly	Glu	Gly	Ala	Ala	Lys	Pro	
	305					310					315					320	
5	Tyr	Thr	Ala	Leu	Ala	Leu	Gln	Thr	Ile	Ser	Arg	His	Phe	Arg	Cys	Leu	
					325					330					335		
	Arg	Asp	Ala	Ile	Cys	Asp	Gln	Ile	Arg	Ala	Ser	Arg	Arg	Ser	Leu	Gly	
				340					345					350			
10	Glu	Gln	Asp	Ala	Ser	Glu	Asn	Ser	Lys	Ala	Ile	Gly	Ile	Ser	Arg	Leu	
			355					360					365				
	Arg	Phe	Val	Asp	His	His	Ile	Arg	Gln	Gln	Arg	Ala	Leu	Gln	Gln	Leu	
15		370					375					380					
	Gly	Met	Met	Gln	Gln	His	Ala	Trp	Arg	Pro	Gln	Arg	Gly	Leu	Pro	Glu	
	385					390					395					400	
20	Ser	Ser	Val	Ser	Val	Leu	Arg	Ala	Trp	Leu	Phe	Glu	His	Phe	Leu	His	
					405					410					415		
	Pro	Tyr	Pro	Lys	Asp	Ser	Asp	Lys	Ile	Met	Leu	Ala	Arg	Gln	Thr	Gly	
				420					425					430			
25	Leu	Thr	Arg	Ser	Gln	Val	Ser	Asn	Trp	Phe	Ile	Asn	Ala	Arg	Val	Arg	
			435					440					445				
	Leu	Trp	Lys	Pro	Met	Val	Glu	Glu	Met	Tyr	Lys	Glu	Glu	Ala	Gly	Asp	
30		450					455					460					
	Ala	Lys	Ile	Asp	Ser	Asn	Ser	Ser	Ser	Asp	Val	Ala	Pro	Arg	Leu	Ala	
	465					470					475					480	
35	Thr	Lys	Asp	Ser	Lys	Val	Glu	Glu	Arg	Gly	Glu	Leu	His	Gln	Asn	Ala	
					485					490					495		
	Ala	Ser	Glu	Phe	Glu	Gln	Tyr	Asn	Ser	Gly	Gln	Ile	Leu	Glu	Ser	Lys	
				500					505					510			
40	Ser	Asn	His	Glu	Ala	Asp	Val	Glu	Met	Glu	Gly	Ala	Ser	Asn	Ala	Glu	
			515					520					525				
	Thr	Gln	Ser	Gln	Ser	Gly	Met	Glu	Asn	Gln	Thr	Gly	Glu	Pro	Leu	Pro	
45		530					535					540					
	Ala	Met	Asp	Asn	Cys	Thr	Leu	Phe	Gln	Asp	Ala	Phe	Val	Gln	Ser	Asn	
	545					550					555					560	
50	Asp	Arg	Phe	Ser	Glu	Phe	Gly	Ser	Phe	Gly	Ser	Gly	Asn	Val	Leu	Pro	
					565					570					575		
	Asn	Gly	Val	Ser	Leu	Thr	Leu	Gly	Leu	Gln	Gln	Gly	Glu	Gly	Ser	Asn	
				580				585					590				
55	Leu	Pro	Met	Ser	Ile	Glu	Thr	His	Val	Ser	Tyr	Val	Pro	Leu	Arg	Ala	
			595					600					605				
	Asp	Asp	Met	Tyr	Ser	Thr	Ala	Pro	Thr	Thr	Met	Val	Pro	Glu	Thr	Ala	
60		610					615					620					

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[0069]

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Conserved regions in accordance with the present invention include the homeodomain region (including the proline-tyrosine-proline loop between helices I and II), the amino-terminal SKY box, the BELL domain, and the carboxy-terminal VSLTLGL-box (SEQ ID NO:15), as described in Examples 20-32, below. Thus, one embodiment of the present invention relates to an isolated nucleic acid molecule encoding a protein having at least 85%, preferably 90%, similarity to the homeodomain region, the amino-terminal SKY box, the BELL domain, and the carboxy-terminal VSLTLGL-box (SEQ ID NO:15) in either SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, or SEQ ID NO:14 by basic BLAST using default parameters analysis. Sequences identified using DNASTar Mega alignment program as either variable or conserved in a gene can be amplified using standard PCR methods using forward and reverse primers designed to amplify the region of choice and which include a restriction enzyme sequence to allow ligation of the PCR product into a vector of choice. Combinations of amplified conserved and variable region sequences can be ligated into a single vector to create a "cassette" which contains a plurality of DNA molecules in one vector.

[0074] Mutations or variants of the above polypeptides or proteins are encompassed by the present invention. Variants may be made by, for example, the deletion or addition of amino acids that have minimal influence on the properties, secondary structure, and hydrophobic nature of a polypeptide or protein. For example, a polypeptide may be conjugated to a signal (or leader) sequence at the N-terminal end of the protein which co-translationally or post-translationally directs transfer of the protein. The polypeptide may also be conjugated to a linker or other sequence for ease of synthesis, purification, or identification of the polypeptide.

[0075] Also suitable as an isolated nucleic acid molecule according to the present invention is a nucleic acid molecule having a nucleotide sequence that is at least 55% similar, preferably at least 80% similar, and most preferably, at least 90% similar, to the nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, or SEQ ID NO:13 by basic BLAST using default parameters analysis.



[0076] Suitable nucleic acid molecules are those that hybridize to a nucleic acid molecule comprising a nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, or SEQ ID NO:13 under stringent conditions. For the purposes of defining the level of stringency, reference can conveniently be made to Sambrook et al., Molecular Cloning: a Laboratory Manual, 2<sup>nd</sup> Edition, Cold Spring Harbor, NY, Cold Spring Harbor Laboratory Press, at 11.45 (1989). An example of low stringency conditions is 4-6X SSC/0.1-0.5% w/v SDS at 37°-45°C for 2-3 hours. Depending on the source and concentration of the nucleic acid involved in the hybridization, alternative conditions of stringency may be employed such as medium stringent conditions. Examples of medium stringent conditions include 1-4X SSC/0.25% w/v SDS at  $\geq$  45°C for 2-3 hours. An example of high stringency conditions includes 0.1-1X SSC/0.1% w/v SDS at 60°C for 1-3 hours. The skilled artisan is aware of various parameters which may be altered during hybridization and washing and which will either maintain or change the stringency conditions. Other examples of high stringency conditions include: 4-5X SSC/0.1% w/v SDS at 54° C for 1-3 hours and 4X SSC at 65°C, followed by a washing in 0.1X SSC at 65°C for about one hour. Alternatively, an exemplary stringent hybridization condition is in 50% formamide, 4X SSC, at 42°C. Still another example of stringent conditions include hybridization at 62°C in 6X SSC, .05X BLOTTO, and washing at 2X SSC, 0.1% SDS at 62°C.

[0077] The precise conditions for any particular hybridization are left to those skilled in the art because there are variables involved in nucleic acid hybridizations beyond those of the specific nucleic acid molecules to be hybridized that affect the choice of hybridization conditions. These variables include: the substrate used for nucleic acid hybridization (e.g., charged vs. non-charged membrane); the detection method used (e.g., radioactive vs. chemiluminescent); and the source and concentration of the nucleic acid involved in the hybridization. All of these variables are routinely taken into account by those skilled in the art prior to undertaking a nucleic acid hybridization procedure.

[0078] A BEL transcription factor of the present invention is preferably produced in purified form (e.g., at least about 80%, more preferably 90% pure) by

conventional techniques. For example, a BEL transcription factor of the present invention may be secreted into the growth medium of recombinant host cells. To isolate the BEL transcription factor, a protocol involving a host cell such as *Escherichia coli* may be used, in which protocol the *E. coli* host cell carrying a recombinant plasmid is propagated, homogenized, and the homogenate is centrifuged to remove bacterial debris. The supernatant is then subjected to sequential ammonium sulfate precipitation. The fraction containing the BEL transcription factor of the present invention is subjected to gel filtration in an appropriately sized dextran or polyacrylamide column to separate the proteins or polypeptides. If necessary, the protein fraction may be further purified by high performance liquid chromatography ("HPLC").

[0079] The present invention relates to a DNA construct that contains a DNA molecule encoding for a BEL transcription factor. This involves incorporating one or more of the nucleic acid molecules of the present invention, or a suitable portion thereof, into host cells using conventional recombinant DNA technology. Generally, this involves inserting the nucleic acid molecule into an expression system to which the nucleic acid molecule is heterologous (i.e. not normally present). The expression system contains the necessary elements for the transcription and translation of the inserted protein-coding sequences.

[0080] The present invention also relates to an expression vector containing a nucleic acid molecule encoding a BEL transcription factor of the present invention. The nucleic acid molecules of the present invention may be inserted into any of the many available expression vectors and cell systems using reagents that are well known in the art. In preparing a DNA vector for expression, the various DNA sequences may normally be inserted or substituted into a bacterial plasmid. Any convenient plasmid may be employed, which will be characterized by having a bacterial replication system, a marker which allows for selection in a bacterium, and generally one or more unique, conveniently located restriction sites. Numerous plasmids, referred to as transformation vectors, are available for transformation. The selection of a vector will depend on the preferred transformation technique and target cells for transfection.

[0081] Suitable vectors include, but are not limited to, the following viral vectors such as lambda vector system gt11, gt WES.tB, Charon 4, and plasmid vectors such as pBR322, pBR325, pACYC177, pACYC1084, pUC8, pUC9, pUC18, pUC19, pLG339, pR290, pKC37, pKC101, SV 40, pBluescript II SK +/- or KS +/- (see "Stratagene Cloning Systems" Catalog (1993) from Stratagene, La Jolla, CA, which is hereby incorporated by reference in its entirety), pQE, pIH821, pGEX, pET series (see F.W. Studier et. al., "Use of T7 RNA Polymerase to Direct Expression of Cloned Genes," Gene Expression Technology vol. 185 (1990), which is hereby incorporated by reference in its entirety), pCB201, and any derivatives thereof. Any appropriate vectors now known or later described for genetic transformation are suitable for use with the present invention. Recombinant molecules can be introduced into cells via transformation, particularly transduction, conjugation, mobilization, or electroporation. The DNA sequences are cloned into the vector using standard cloning procedures in the art, as described by Sambrook et al., Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor Press, NY (1989), and Ausubel, F. M. et al. (1989) Current Protocols in Molecular Biology, John Wiley & Sons, New York, N.Y., which are hereby incorporated by reference in their entirety.

[0082] U.S. Patent No. 4,237,224 issued to Cohen and Boyer, which is hereby incorporated by reference in its entirety, describes the production of expression systems in the form of recombinant plasmids using restriction enzyme cleavage and ligation with DNA ligase. These recombinant plasmids are then introduced by means of transformation and replicated in unicellular cultures including prokaryotic organisms and eukaryotic cells grown in tissue culture.

[0083] A variety of host-vector systems may be utilized to express the protein-encoding sequence(s). Primarily, the vector system must be compatible with the host cell used. Host-vector systems include but are not limited to the following: bacteria transformed with bacteriophage DNA, plasmid DNA, or cosmid DNA; microorganisms such as yeast containing yeast vectors; mammalian cell systems infected with virus (e.g., vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (e.g., baculovirus); and plant cells infected by bacteria. The expression elements of these vectors vary in their strength and

specificities. Depending upon the host-vector system utilized, any one of a number of suitable transcription and translation elements can be used.

[0084] Thus, certain "control elements" or "regulatory sequences" are also incorporated into the plasmid-vector constructs of the present invention. These  
5 include non-transcribed regions of the vector and 5' and 3' untranslated regions, which interact with host cellular proteins to carry out transcription and translation. Such elements may vary in their strength and specificity. Depending on the vector system and host utilized, any number of suitable transcription and/or translation elements, including constitutive, inducible, and repressible promoters, as well as  
10 minimal 5' promoter elements may be used. A constitutive promoter is a promoter that directs expression of a gene throughout the development and life of an organism. An inducible promoter is a promoter that is capable of directly or indirectly activating transcription of one or more DNA sequences or genes in response to an inducer. In the absence of an inducer, the DNA sequences or genes  
15 will not be transcribed or will only be minimally transcribed.

[0085] The DNA sequences of eukaryotic promoters differ from those of prokaryotic promoters. Furthermore, eukaryotic promoters and accompanying genetic signals may not be recognized in or may not function in a prokaryotic system, and, further, prokaryotic promoters are not recognized and do not function  
20 in eukaryotic cells.

[0086] Promoters vary in their "strength" (i.e. their ability to promote transcription). For the purposes of expressing a cloned gene, it is desirable to use strong promoters in order to obtain a high level of transcription and, hence, expression of the gene. Depending upon the host cell system utilized, any one of  
25 a number of suitable promoters may be used. For instance, when cloning in *E. coli*, its bacteriophages, or plasmids, promoters such as the T7 phage promoter, *lac* promoter, *trp* promoter, *recA* promoter, ribosomal RNA promoter, the P<sub>R</sub> and P<sub>L</sub> promoters of coliphage lambda and others, including but not limited, to *lacUV5*, *ompF*, *bla*, *lpp*, and the like, may be used to direct high levels of  
30 transcription of adjacent DNA segments. Additionally, a hybrid *trp-lacUV5 (tac)* promoter or other *E. coli* promoters produced by recombinant DNA or other

synthetic DNA techniques may be used to provide for transcription of the inserted gene.

[0087] Other examples of some constitutive promoters that are widely used for inducing expression of transgenes include the nopoline synthase (NOS) gene promoter, from *Agrobacterium tumefaciens*, (U.S. Patent No. 5,034,322 issued to Rogers et al., which is hereby incorporated by reference in its entirety), the cauliflower mosaic virus (CaMV) 35S and 19S promoters (U.S. Patent No. 5,352,605 issued to Fraley et al., which is hereby incorporated by reference in its entirety), the enhanced CaMV35S promoter ("enh CaMV35S"), the figwort mosaic virus full-length transcript promoter ("FMV35S"), those derived from any of the several actin genes, which are known to be expressed in most cells types (U.S. Patent No. 6,002,068 issued to Privalle et al., which is hereby incorporated by reference in its entirety), and the ubiquitin promoter, which is a gene product known to accumulate in many cell types. Examples of constitutive promoters for use in mammalian cells include the RSV promoter derived from Rous sarcoma virus, the CMV promoter derived from cytomegalovirus,  $\beta$ -actin and other actin promoters, and the EF1 $\alpha$  promoter derived from the cellular elongation factor 1 $\alpha$  gene.

[0088] Bacterial host cell strains and expression vectors may be chosen which inhibit the action of the promoter unless specifically induced. In certain operations, the addition of specific inducers is necessary for efficient transcription of the inserted nucleic acid. For example, the *lac* operon is induced by the addition of lactose or IPTG (isopropylthio-beta-D-galactoside). A variety of other operons, such as *trp*, *pro*, etc., are under different controls.

[0089] Other examples of some inducible promoters, induced, for examples by a chemical agent, such as a metabolite, growth regulator, herbicide or phenolic compound, or a physiological stress/physical means, such as cold, heat, salt, toxins, or through the action of a pathogen or disease agent such as a virus or fungus, include a glucocorticoid-inducible promoter (Schena et al., Proc. Natl. Acad. Sci. 88:10421-5 (1991), which is hereby incorporated by reference in its entirety), the heat shock promoter ("Hsp"), IPTG or tetracycline ("Tet on" system), the metallothionine promoter, which is activated by heavy metal ions,

and hormone-responsive promoters, which are activated by treatment of certain hormones. A host cell containing an inducible promoter may be exposed to an inducer by externally applying the inducer to the cell. In addition, "tissue-specific" promoters can be used, which are promoters that function in a tissue specific manner to regulate the gene of interest within selected tissues of the host. Examples of such tissue specific promoters include seed, flower, or root specific promoters as are well known in the field (e.g., U.S. Patent No. 5,750,385 to Shewmaker et al., which is hereby incorporated by reference in its entirety). Promoters of the nucleic acid construct of the present invention may be either homologous (derived from the same species as the host cell) or heterologous (derived from a different species than the host cell).

[0090] Specific initiation signals are also required for efficient gene transcription and translation in prokaryotic cells. These transcription and translation initiation signals may vary in "strength" as measured by the quantity of gene specific messenger RNA and protein synthesized, respectively. The DNA expression vector, which contains a promoter, may also contain any combination of various "strong" transcription and/or translation initiation signals. For instance, efficient translation in *E. coli* requires an SD sequence about 7-9 bases 5' to the initiation codon ("ATG") to provide a ribosome binding site. Thus, any SD-ATG combination that can be utilized by host cell ribosomes may be employed. Such combinations include but are not limited to the SD-ATG combination from the *cro* gene or the *N* gene of coliphage lambda, or from the *E. coli* tryptophan E, D, C, B or A genes. Additionally, any SD-ATG combination produced by recombinant DNA or other techniques involving incorporation of synthetic nucleotides may be used.

[0091] The constructs of the present invention also include an operable 3' regulatory region, selected from among those which are capable of providing correct transcription termination and polyadenylation of mRNA for expression in the host cell of choice, operably linked to a DNA molecule which encodes for a protein of choice. A number of 3' regulatory regions are known in the art. Virtually any 3' regulatory region known to be operable in the host cell of choice

would suffice for proper expression of the coding sequence of the nucleic acid of the present invention.

[0092] In one aspect of the present invention, the nucleic acid molecule of the present invention is incorporated into an appropriate vector in the sense  
5 direction, such that the open reading frame is properly oriented for the expression of the encoded protein under control of a promoter of choice. This involves the inclusion of the appropriate regulatory elements into the DNA-vector construct. These include non-translated regions of the vector, useful promoters, and 5' and 3' untranslated regions which interact with host cellular proteins to carry out  
10 transcription and translation. Such elements may vary in their strength and specificity. Depending on the vector system and host utilized, any number of suitable transcription and translation elements, including constitutive and inducible promoters, may be used.

[0093] A nucleic acid molecule of the present invention, promoter of  
15 choice, an appropriate 3' regulatory region, and, if desired, a reporter gene, can be incorporated into a vector-expression system to contain a nucleic acid of the present invention, or a suitable fragment thereof, using standard cloning techniques as described in Sambrook et al., Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor Press, NY (1989), and Ausubel et al.  
20 (1989) Current Protocols in Molecular Biology, John Wiley & Sons, New York, N.Y., which are hereby incorporated by reference in their entirety. The transcriptional and translational elements are operably linked to the nucleic acid molecule of the present invention or a fragment thereof, meaning that the resulting vector expresses the BEL transcription factor when placed in a suitable host cell.

[0094] Once an isolated DNA molecule encoding a BEL transcription  
25 factor has been cloned into an expression vector, it is ready to be incorporated into a host cell. Such incorporation can be carried out by the various forms of transformation noted above, depending upon the vector/host cell system. Recombinant molecules can be introduced into cells via transformation,  
30 particularly transduction, conjugation, mobilization, or electroporation. The nucleic acid sequences are cloned into the host cell using standard cloning procedures known in the art, as described by Sambrook et al., Molecular Cloning:

A Laboratory Manual, Second Edition, Cold Springs Laboratory, Cold Springs Harbor, New York (1989), which is hereby incorporated by reference in its entirety. Suitable host cells include, but are not limited to, bacteria, virus, yeast, mammalian cells, insect, plant, and the like.

5    [0095]       Thus, the present invention also relates to a host cell incorporating one or more of the isolated nucleic acid molecules of the present invention. In one embodiment, the isolated nucleic acid molecule is heterologous to the host cell. Such incorporation can be carried out by the various forms of transformation noted above, depending upon the vector/host system, and using the various host  
10   cells described above.

      [0096]       Methods of transformation may result in transient or stable expression of the DNA under control of the promoter. Preferably, the nucleic acid of the present invention is stably inserted into the genome of the host cell as a result of the transformation, although transient expression can serve an important  
15   purpose.

      [0097]       One approach to transforming host cells with a nucleic acid molecule of the present invention is particle bombardment (also known as biolistic transformation) of the host cell. This can be accomplished in one of several ways. The first involves propelling inert or biologically active particles at cells. This  
20   technique is disclosed in U.S. Patent Nos. 4,945,050, 5,036,006, and 5,100,792, all to Sanford et al., which are hereby incorporated by reference in their entirety. Generally, this procedure involves propelling inert or biologically active particles at the cells under conditions effective to penetrate the outer surface of the cell and to be incorporated within the interior thereof. When inert particles are utilized,  
25   the vector can be introduced into the cell by coating the particles with the vector containing the heterologous DNA. Alternatively, the target cell can be surrounded by the vector so that the vector is carried into the cell by the wake of the particle. Biologically active particles (e.g., dried bacterial cells containing the vector and heterologous DNA) can also be propelled into plant cells. Other variations of  
30   particle bombardment, now known or hereafter developed, can also be used.

      [0098]       Transient expression in protoplasts allows quantitative studies of gene expression, because the population of cells is very high (on the order of  $10^6$ ).



To deliver DNA inside protoplasts, several methodologies have been proposed, but the most common are electroporation (Fromm et al., Proc. Natl. Acad. Sci. USA 82:5824-5828 (1985), which is hereby incorporated by reference in its entirety) and polyethylene glycol (PEG) mediated DNA uptake (Krens et al.,  
5 Nature 296:72-74 (1982), which is hereby incorporated by reference in its entirety). During electroporation, the DNA is introduced into the cell by means of a reversible change in the permeability of the cell membrane due to exposure to an electric field. PEG transformation introduces the DNA by changing the elasticity of the membranes. Unlike electroporation, PEG transformation does not require  
10 any special equipment and transformation efficiencies can be equally high. Another appropriate method of introducing the nucleic acid molecule of the present invention into a host cell is fusion of protoplasts with other entities, either minicells, cells, lysosomes, or other fusible lipid-surfaced bodies that contain the chimeric gene (Fraley, et al., Proc. Natl. Acad. Sci. USA 76:3348-52 (1979),  
15 which is hereby incorporated by reference in its entirety).

[0099] Stable transformants are preferable for the methods of the present invention. An appropriate method of stably introducing the nucleic acid molecule into plant cells is to infect a plant cell with *Agrobacterium tumefaciens* or *Agrobacterium rhizogenes* previously transformed with a DNA construct of the  
20 present invention. Under appropriate conditions known in the art, the transformed plant cells are grown to form shoots or roots, and develop further into plants.

[0100] Plant tissues suitable for transformation include without limitation, floral buds, leaf tissue, root tissue, meristems, zygotic and somatic embryos, megaspores, callus, protoplasts, tassels, pollen, embryos, anthers, and the like.  
25 The means of transformation chosen is that most suited to the tissue to be transformed.

[0101] Suitable plants include dicots and monocots. Monocots suitable for the present invention include Gramineae (e.g., grass, corn, grains, bamboo, sugar cane), Liliaceae (e.g., onion, garlic, asparagus, tulips, hyacinths, day lily, and  
30 aloes), Iridaceae (e.g., iris, gladioli, freesia, crocus, and watsonia), and Orchidacea (e.g., orchid). Examples of dicots suitable for the present invention include Salicaceae (e.g., willow, and poplar), Ranunculaceae (e.g., *Delphinium*, *Paeonia*,

*Ranunculus*, *Anemone*, *Clematis*, columbine, and marsh marigold), Magnoliaceae (e.g., tulip tree and *Magnolia*), Cruciferae (e.g., mustards, cabbage, cauliflower, broccoli, brussel sprouts, kale, kohlrabi, turnip, and radish), Rosaceae (e.g., strawberry, blackberry, peach, apple, pear, quince, cherry, almond, plum, apricot, and rose), Leguminosae (e.g., pea, bean, peanut, alfalfa, clover, vetch, redbud, broom, wisteria, lupine, black locust, and acacia), Malvaceae (e.g., cotton, okra, and mallow), Umbelliferae (e.g., carrot, parsley, parsnips, and hemlock), Labiatae (e.g., mint, peppermints, spearmint, thyme, sage, and lavender), Solanaceae (e.g., potato, tomato, pepper, eggplant, tobacco, henbane, atropa, physalis, datura, and *Petunia*), Cucurbitaceae (e.g., melon, squash, pumpkin, and cucumber), Compositae (e.g., sunflower, endive, artichoke, lettuce, safflower, aster, marigold, dandelions, sage brush, *Dalia*, *Chrysanthemum*, and *Zinna*), and Rubiaceae (e.g., coffee).

[0102] After transformation, the transformed plant cells can be selected and regenerated. Preferably, transformed cells are first identified using a selection marker simultaneously introduced into the host cells along with the DNA construct of the present invention. Suitable selection markers include, without limitation, markers encoding for antibiotic resistance, such as the *nptII* gene which confers kanamycin resistance (Fraley, et al., Proc. Natl. Acad. Sci. USA 80:4803-4807 (1983), which is hereby incorporated by reference in its entirety), and the genes which confer resistance to gentamycin, G418, hygromycin, streptomycin, spectinomycin, tetracycline, chloramphenicol, and the like. Any known antibiotic-resistance marker can be used to transform and select transformed host cells in accordance with the present invention. Cells or tissues are grown on a selection medium containing the appropriate antibiotic, whereby generally only those transformants expressing the antibiotic resistance marker continue to grow. Other types of markers are also suitable for inclusion in the expression cassette of the present invention. For example, a gene encoding for herbicide tolerance, such as tolerance to sulfonylurea is useful, or the *dhfr* gene, which confers resistance to methotrexate (Bourouis et al., EMBO J. 2:1099-1104 (1983), which is hereby incorporated by reference in its entirety). Similarly, "reporter genes," which encode for enzymes providing for production of a compound identifiable are

suitable. The most widely used reporter gene for gene fusion experiments has been *uidA*, a gene from *Escherichia coli* that encodes the  $\beta$ -glucuronidase protein, also known as GUS (Jefferson et al., EMBO J. 6:3901-3907 (1987), which is hereby incorporated by reference in its entirety). Similarly, enzymes providing  
5 for production of a compound identifiable by luminescence, such as luciferase, are useful. The selection marker employed will depend on the target species; for certain target species, different antibiotics, herbicide, or biosynthesis selection markers are preferred.

[0103] Once a recombinant plant cell or tissue has been obtained, it is  
10 possible to regenerate a full-grown plant therefrom. It is known that practically all plants can be regenerated from cultured cells or tissues. Means for regeneration vary from species to species of plants, but generally a suspension of transformed protoplasts or a petri plate containing transformed explants is first provided. Callus tissue is formed and shoots may be induced from callus and subsequently  
15 rooted. Alternatively, embryo formation can be induced in the callus tissue. These embryos germinate as natural embryos to form plants. The culture media will generally contain various amino acids and hormones, such as auxin and cytokinins. It is also advantageous to add glutamic acid and proline to the medium, especially for such species as corn and alfalfa. Efficient regeneration  
20 will depend on the medium, on the genotype, and on the history of the culture. If these three variables are controlled, then regeneration is usually reproducible and repeatable.

[0104] Plant regeneration from cultured protoplasts is described in Evans, et al., Handbook of Plant Cell Cultures, Vol. 1: (MacMillan Publishing Co., New  
25 York, 1983); and Vasil I.R. (ed.), Cell Culture and Somatic Cell Genetics of Plants, Acad. Press, Orlando, Vol. I, 1984, and Vol. III (1986), which are hereby incorporated by reference in their entirety.

[0105] After the DNA construct is stably incorporated in transgenic plants, it can be transferred to other plants by sexual crossing or by preparing cultivars.  
30 With respect to sexual crossing, any of a number of standard breeding techniques can be used depending upon the species to be crossed. Cultivars can be propagated in accord with common agricultural procedures known to those in the

field. Alternatively, transgenic seeds or propagules (e.g., cuttings) are recovered from the transgenic plants. The seeds can then be planted in the soil and cultivated using conventional procedures to produce transgenic plants.

- [0106] The present invention is also directed to a method for enhancing  
5 tuber development in a plant. This method includes transforming a tuberous plant with a first DNA construct including a first nucleic acid molecule encoding a BEL transcription factor or a KNOX transcription factor, and a first operably linked promoter and first 3' regulatory region, whereby tuber development in the plant is enhanced.
- 10 [0107] Suitable BEL transcription factors include BEL transcription factors from potato, as described above. Other suitable BEL transcription factors include, but are not limited to, those from tobacco, tomato (see, e.g., GenBank Accession Nos. AF375964, AF375965, and AF375966), Arabidopsis, rice, barley, apple, and bago (*Gnetum gnemon*).
- 15 [0108] As used herein, a KNOX transcription factor is encoded by a *Knotted*-like homeobox (*knox*) gene and includes a KNOX domain. KNOX transcription factors regulate growth, in particular, leaf architecture and meristem growth. KNOX transcription factors have been isolated from several plant species (reviewed in Reiser et al., "Knots in the Family Tree: Evolutionary Relationships  
20 and Functions of *knox* Homeobox Genes," Plant Mol. Biol. 42:151-166 (2000), which is hereby incorporated by reference in its entirety) and can be divided into two classes based on expression patterns and sequence similarity (Kerstetter et al., "Sequence Analysis and Expression Patterns Divide the Maize *knotted1*-like Homeobox Genes into Two Classes," Plant Cell 6:1877-1887 (1994), which is  
25 hereby incorporated by reference in its entirety). Class I *knox* genes have high similarity to the maize *knotted1* (*kn1*) homeodomain and generally have a meristem-specific mRNA expression pattern. Class II *knox* genes usually have a more widespread expression pattern. *Knox* genes are members of the three amino acid loop extension (TALE) superclass of homeobox genes (Bürglin, "Analysis of  
30 TALE Superclass Homeobox Genes (MEIS, PBC, KNOX, Iroquois, TGIF) Reveals a Novel Domain Conserved Between Plants and Animals," Nucleic Acids Res 25:4173-4180 (1997), which is hereby incorporated by reference in its

entirety). *Knox* genes share conserved regions outside of the homeodomain including the MEINOX and ELK domains.

[0109] Suitable KNOX transcription factors include, but are not limited to, POTH1, POTH15, POTH2, HO9, NTH Types (1, 9, 15, 20, 22) (Nishamura et al., 5 Plant J. 18:337-347 (1999), which is hereby incorporated by reference in its entirety), those from *Arabidopsis*, maize, barley, tobacco, tomato, pea, cabbage, *Ipomoea*, *Helianthus*, *Medicago*, and *Dendrobium*.

[0110] In one embodiment, the KNOX transcription factor is POTH1 and is encoded by a nucleic acid molecule having a nucleotide sequence of SEQ ID 10 NO:16 as follows:

```
1 gagtttctct cccttttaaa aaagaaaaaa aaaacacaac acccacttca aatatcaaac
61 aaatttctca tttgattatt tctaagtgat ttacactact ttgtattttt gtttgttttt
121 ttttagatat atatatggat gatgaaatgt atgggttttca ttcaacaaga gacgattacg
15 181 cggataaagc tttgatgtca ccggagaatt tgatgatgca aactgagtac aacaatttcc
241 acaactatac caactcgtcc atcttgactt ctaatccgat gatgttttga tccgatgata
301 ttcaattatc atcgggaacaa actaatttct tcagtactat gactcttcaa aataatgata
361 atatttatca aattagaagt ggaattgtg gcggaggcag tggcagtggt ggtagcagta
421 aggatcataa tgataataac aataataatg aagattatga tgaagatggt tcaaatgtta
20 481 tcaaggctaa aatcgtctca catccttatt atcctaattt actcaacgct tatattgatt
541 gccaaaaggt tggagcacca gcgggtatag taaatctgct ggaagaaata aggcaacaaa
601 ctgattttcg taaaccaaac gctacttcta tatgtatagg agctgacocct gaacttgatg
661 agtttatgga aacgtattgt gatataattgt tgaagtataa gtccgatctg tctaggcctt
721 ttgatgaagc aacaacgttc ctcaacaaga ttgaaatgca actaggtaat ctttgcaaaag
25 781 atgatggtgg tgtatcatca gatgaggagt taagttgtgg tgaggcagat gcatcaatga
841 gaagtgaagga taatgaactc aaagatagac tcctacgtaa gtttggaagt catttaagta
901 gtctaaagtt ggaattttca aagaaaaaga agaaaaggaa gctaccaaaa gaggcaaggc
961 aaatgttact tgcattgtgg gatgatcact ttagatggcc ttaccctacg gaggctgata
1021 agaattcact agcagaatca acaggacttg atccaaagca gatcaacaat tgggttataa
1081 atcaaaaggaa gagacattgg aaaccatcag agaatatgca gttagctgtt atggataatc
30 1141 taagctctca gttcttctca tcagatgatt gagtttgaat ggaaattgtg aaaatactgc
1201 tcttcatttc tctttttatt atataataa tataaatagt atatttttgg gaaagaaaga
1261 agttatttta ttaatcaaaa tctctataaa taatggtaga gattaattaa tgttgaattc
1321 ttcttgatca tgtaaatatt caatctagct aattgtcaaa attaatgctt acctaaaaaa
35 1381 aaa
```

The cDNA (Genbank Accession # U65648) includes an open reading frame of 1035 nt coding for a 345-residue protein estimated to have a mass of 37.95 kDa having an amino acid sequence corresponding to SEQ ID NO:17 as follows:

```
40
Met Asp Asp Glu Met Tyr Gly Phe His Ser Thr Arg Asp Asp Tyr Ala
1 5 10 15
Asp Lys Ala Leu Met Ser Pro Glu Asn Leu Met Met Gln Thr Glu Tyr
45 20 25 30
Asn Asn Phe His Asn Tyr Thr Asn Ser Ser Ile Leu Thr Ser Asn Pro
35 40 45
```

	Met	Met	Phe	Gly	Ser	Asp	Asp	Ile	Gln	Leu	Ser	Ser	Glu	Gln	Thr	Asn
	50						55					60				
5	Ser	Phe	Ser	Thr	Met	Thr	Leu	Gln	Asn	Asn	Asp	Asn	Ile	Tyr	Gln	Ile
	65					70					75					80
	Arg	Ser	Gly	Asn	Cys	Gly	Gly	Gly	Ser	Gly	Ser	Gly	Gly	Ser	Ser	Lys
					85					90					95	
10	Asp	His	Asn	Asp	Asn	Asn	Asn	Asn	Asn	Glu	Asp	Tyr	Asp	Glu	Asp	Gly
				100					105					110		
	Ser	Asn	Val	Ile	Lys	Ala	Lys	Ile	Val	Ser	His	Pro	Tyr	Tyr	Pro	Lys
15			115					120					125			
	Leu	Leu	Asn	Ala	Tyr	Ile	Asp	Cys	Gln	Lys	Val	Gly	Ala	Pro	Ala	Gly
	130						135					140				
20	Ile	Val	Asn	Leu	Leu	Glu	Glu	Ile	Arg	Gln	Gln	Thr	Asp	Phe	Arg	Lys
	145					150					155					160
	Pro	Asn	Ala	Thr	Ser	Ile	Cys	Ile	Gly	Ala	Asp	Pro	Glu	Leu	Asp	Glu
					165					170					175	
25	Phe	Met	Glu	Thr	Tyr	Cys	Asp	Ile	Leu	Leu	Lys	Tyr	Lys	Ser	Asp	Leu
				180					185					190		
	Ser	Arg	Pro	Phe	Asp	Glu	Ala	Thr	Thr	Phe	Leu	Asn	Lys	Ile	Glu	Met
30			195					200					205			
	Gln	Leu	Gly	Asn	Leu	Cys	Lys	Asp	Asp	Gly	Gly	Val	Ser	Ser	Asp	Glu
	210						215					220				
35	Glu	Leu	Ser	Cys	Gly	Glu	Ala	Asp	Ala	Ser	Met	Arg	Ser	Glu	Asp	Asn
	225					230					235					240
	Glu	Leu	Lys	Asp	Arg	Leu	Leu	Arg	Lys	Phe	Gly	Ser	His	Leu	Ser	Ser
					245					250					255	
40	Leu	Lys	Leu	Glu	Phe	Ser	Lys	Lys	Lys	Lys	Lys	Gly	Lys	Leu	Pro	Lys
				260					265					270		
	Glu	Ala	Arg	Gln	Met	Leu	Leu	Ala	Trp	Trp	Asp	Asp	His	Phe	Arg	Trp
45				275				280					285			
	Pro	Tyr	Pro	Thr	Glu	Ala	Asp	Lys	Asn	Ser	Leu	Ala	Glu	Ser	Thr	Gly
	290						295					300				
50	Leu	Asp	Pro	Lys	Gln	Ile	Asn	Asn	Trp	Phe	Ile	Asn	Gln	Arg	Lys	Arg
	305					310					315					320
	His	Trp	Lys	Pro	Ser	Glu	Asn	Met	Gln	Leu	Ala	Val	Met	Asp	Asn	Leu
					325					330					335	
55	Ser	Ser	Gln	Phe	Phe	Ser	Ser	Asp	Asp							
				340					345							

[0111] In accordance with the present invention, the BEL or KNOX transcription factor may be expressed throughout the plant to achieve enhanced tuber development (see Examples below). Alternatively, the BEL or KNOX transcription factor may be expressed in an organ-specific manner. This is  
5 beneficial when, for example with POTH1, expression throughout the plant results in dwarf transgenic plants with altered leaf morphology. In these circumstances, specific expression in the stolon, for example, may be desirable.

[0112] In one embodiment of this method of the present invention, the tuberous plant is transformed with one or more DNA constructs which include  
10 nucleic acid molecules encoding both a BEL transcription factor and a KNOX transcription factor. Alternatively, a plant expressing one or more of a BEL transcription factor or a KNOX transcription factor may be transformed with a DNA construct including a nucleic acid molecule encoding only one of a BEL transcription factor or a KNOX transcription factor.

15 [0113] Tuberous plants suitable for use in this method of the present invention include potato, dahlia, caladium, Jerusalem artichoke (*Helianthus tuberosus*), yam (*Dioscorea alata*), sweet potato (*Ipomoea batatas*), cassava (*Manihot esculenta*), tuberous begonia, cyclamen, and other solanum species (e.g., wild potato).

20 [0114] Another aspect of the present invention relates to a method of enhancing growth in a plant. This method includes transforming a plant with a DNA construct including a nucleic acid molecule encoding a BEL transcription factor from *Solanum tuberosum* and an operably linked promoter and 3' regulatory region, whereby growth in the plant is enhanced.

25 [0115] Suitable plants which may be transformed in this method of the present invention include Gramineae (e.g., grass, corn, grains, bamboo, sugar cane), Liliaceae (e.g., onion, garlic, asparagus, tulips, hyacinths, day lily, and aloes), Iridaceae (e.g., iris, gladioli, freesia, crocus, and watsonia), Orchidaceae (e.g., orchid), Salicaceae (e.g., willow, and poplar), Ranunculaceae (e.g.,  
30 *Delphinium*, *Paeonia*, *Ranunculus*, *Anemone*, *Clematis*, columbine, and marsh marigold), Magnoliaceae (e.g., tulip tree and *Magnolia*), Cruciferae (e.g., mustards, cabbage, cauliflower, broccoli, brussel sprouts, kale, kohlrabi, turnip,

and radish), Rosaceae (e.g., strawberry, blackberry, peach, apple, pear, quince, cherry, almond, plum, apricot, and rose), Leguminosae (e.g., pea, bean, peanut, alfalfa, clover, vetch, redbud, broom, wisteria, lupine, black locust, and acacia), Malvaceae (e.g., cotton, okra, and mallow), Umbelliferae (e.g., carrot, parsley, 5 parsnips, and hemlock), Labiatae (e.g., mint, peppermints, spearmint, thyme, sage, and lavender), Solanaceae (e.g., potato, tomato, pepper, eggplant, tobacco, henbane, atropa, physalis, datura, and *Petunia*), Cucurbitaceae (e.g., melon, squash, pumpkin, and cucumber), Compositae (e.g., sunflower, endive, artichoke, lettuce, safflower, aster, marigold, dandelions, sage brush, *Dalia*, *Chrysanthemum*, 10 and *Zinna*), and Rubiaceae (e.g., coffee). In one particular embodiment, the plant transformed is a solanaceous species.

[0116] Yet another embodiment of the present invention relates to a method of regulating flowering in a plant. This method includes transforming a plant with a DNA construct including a nucleic acid molecule encoding a BEL 15 transcription factor from *Solanum tuberosum* and an operably linked promoter and 3' regulatory region, whereby flowering in the plant is regulated.

[0117] Suitable plants in accordance with this method of the present invention are described above.

[0118] The BEL transcription factors from *Solanum tuberosum* of the 20 present invention appear to play a diverse role in plant growth by regulating the development of both reproductive and vegetative meristems. Accordingly, they can be used in the methods for enhancing growth or regulating flowering of the present invention. In particular, the BEL transcription factors of the present invention are involved in regulating photoperiodic responses in potato 25 (tuberization), and BEL transcription factors have previously been identified as contributing to flower development (Müller et al., "In vitro Interactions Between Barley TALE Homeodomain Proteins Suggest a Role for Protein-Protein Associations in the Regulation of Knox Gene Function," *Plant J.* 27:13-23 (2001); Mondrusan et al., "Homeotic Transformation of Ovules into Carpel-Like 30 Structures in *Arabidopsis*," *Plant Cell* 6:333-349 (1994); Reiser et al., "The BELL1 Gene Encodes a Homeodomain Protein Involved in Patterns Formation in the *Arabidopsis* Ovule Primordium," *Cell* 83:735-742 (1995), which are hereby



incorporated by reference in their entirety) and are present in numerous photoperiodic flowering species (e.g., rice, tobacco, morning glory, *Arabidopsis*), thus it appears that they contribute to regulating flower induction in many plants.

5

## EXAMPLES

### Example 1 – Amplification of Potato Homeobox Fragment for Use as Probe

[0119] Two primers, Primer 1 (5'-AAGAAGAAGAAGAAAGGGAA) (SEQ ID NO:18) and Primer 2 (5'-ATGAACCAGTTGTTGAT) (SEQ ID NO:19) were designed based on comparison of the homeobox regions of five class I homeobox genes (*KN1*, *KNAT1*, *KNAT2*, *OSHI*, and *SBH1*) to correspond to the most highly conserved portions of the homeobox, and were synthesized at the DNA Synthesis Facility at Iowa State University. Template DNA was prepared from a mass *in vivo* excision of a 4-day axillary bud tuber  $\lambda$ ZAP®II cDNA library (Stratagene, La Jolla, CA) from potato cv. Superior. The potato homeobox fragment was amplified using an annealing temperature of 45 °C and cloned into the pCR2.1 vector of the TA Cloning® Kit (Invitrogen, Carlsbad, CA).

10

15

### Example 2 – Library Screening and Sequence Analysis

[0120] The early tuberization stage library was constructed as described in Kang et al., "A Novel MADS-box Gene of Potato (*Solanum tuberosum* L.) Expressed During the Early Stages of Tuberization," *Plant Mol. Biol.* 31: 379-386 (1996), which is hereby incorporated by reference in its entirety. Screening of 400,000 pfu was accomplished using 100 ng of <sup>32</sup>P-labeled PCR-generated probe in 50 % formamide (50% deionized formamide, 6× SSC, 3.4× Denhardt's solution, 25 mM sodium phosphate buffer, pH 7.0, 120 µg/ml denatured salmon sperm DNA, 0.4% SDS) at 42 °C for 48 hours. Membranes were washed with 2X SSC/0.1 % SDS, at 25 °C for 5 minutes; then twice with 2X SSPE/0.1 % SDS, at 65 °C for 20 minutes.

25

[0121] *POTH1* was sequenced at the Nucleic Acid Sequencing Facility at Iowa State University. Sequence analyses performed included BLAST (Altschul

30

et al., "Basic Local Alignment Search Tool," J. Mol. Biol. 215:403-410 (1990), which is hereby incorporated by reference in its entirety) and GAP [Genetics Computer Group (GCG), Madison, WI].

5    **Example 3 – RNA Isolation and Northern Blot Analysis**

[0122]        Total RNA was isolated (Dix et al., "*In vivo* Transcriptional Products of the Chloroplast DNA of *Euglena gracilis*," Curr. Genet. 7:265-273 (1983), which is hereby incorporated by reference in its entirety) from potato (*Solanum tuberosum* L.) plants grown in the greenhouse at 20 to 25 °C under 16  
10    hours of light. Total RNA was enriched for poly (A)+ RNA by separation over an oligo-dT column and northern gel electrophoresis was performed using methyl mercury as a denaturant. Ethidium bromide staining under UV light was used to ascertain equal gel loading and efficient transfer to nylon membranes. The Genius™ nonradioactive nucleic acid labeling and detection system (Roche  
15    Biochemicals, Indianapolis, IN) was used. Fifteen ng/ml of digoxigenin-UTP-labeled antisense RNA probe in 50 % formamide was hybridized to filters at 55 °C overnight. Membranes were washed twice for 5 minutes in 2X SSC, 0.1 % SDS at 25 °C, and then washed twice for 15 minutes in 0.1X SSC, 0.1 % SDS at 68 °C. The membranes were then incubated 30 minutes in blocking  
20    solution: maleic acid buffer pH 7.5 (1:10), 30 minutes in anti-digoxigenin-alkaline-phosphatase conjugate: maleic acid buffer (1:10,000), washed twice for 15 minutes in maleic acid buffer, and equilibrated 5 minutes in detection buffer before addition of disodium 3-[4-methoxy-1,2-dioxetane-3,2'-[5'-chloro]tricyclo [3.3.1.1<sup>3,7</sup>]decan}-4-yl] phenyl phosphate (CSPD) substrate  
25    solution. Membranes were exposed to film for 30 to 45 minutes at 25 °C.

**Example 4 – In situ Hybridization Analysis**

[0123]        Preparation of tissue samples and *in situ* hybridizations were performed as described in Cañas et al., "Nuclear Localization of the Petunia  
30    MADS Box Protein FBP1," Plant J. 6:597-604 (1994), which is hereby incorporated by reference in its entirety. Digoxigenin-UTP-labeled RNA probes,

both sense and antisense, were transcribed with RNA polymerases according to instructions (Roche Biochemicals, Indianapolis, IN), and hydrolyzed using 0.2 M sodium carbonate and 0.2 M sodium bicarbonate at 65 °C for 51 minutes.

Unincorporated nucleotides were removed over a Sephadex G-50 column.

- 5    **[0124]**        For immunological detection, the slides were incubated in buffer 1 (1 % blocking solution, 100 mM Tris pH 7.5, 150 mM NaCl) for one hour, then equilibrated with buffer 2 (100 mM Tris pH 7.5, 150 mM NaCl, 0.5 % BSA, and 0.3 % Triton X-100). Tissue sections were then incubated with anti-digoxigenin-alkaline-phosphatase conjugate diluted 1:1000 in buffer 2 in a humidified box for  
10 two hours, then washed three times for 20 minutes in 100 mM Tris pH 7.5, 150 mM NaCl. The tissue sections were equilibrated in buffer 3 (100 mM Tris pH 9.5, 100 mM NaCl, 50 mM MgCl<sub>2</sub>) for 10 minutes, then incubated in 3.2 µg/ml 5-bromo-4-chloro-3-indolyl-phosphate (BCIP):6.6 µg/ml nitro-blue tetrazolium salt (NBT) in buffer 3 in a humidified box for 13 hours (above-ground tissues) or 7  
15 hours (underground tissues). Accumulation of *POTH1* mRNA was visualized as an orange/brown stain under dark field illumination. Sections were viewed and photodocumented using the dark field mode on the Leitz Orthoplan light microscope.

20    **Example 5 – 35S-POTH1 Transformation of Potato Plants**

- [0125]**        The full length *POTH1* cDNA was cloned into the binary vector, pCB201 (Xiang et al., “A Mini Binary Vector Series for Plant Transformation,” Plant Mol. Biol. 40:711-718 (1999), which is hereby incorporated by reference in its entirety) between the CaMV 35S promoter and the *nos* terminator. Two potato  
25 cultivars, *Solanum tuberosum* ssp. *andigena* and cv. FL-1607, were transformed by the *Agrobacterium tumefaciens* (strain GV2260) mediated leaf-disk transformation method (Liu et al., “Transformation of *Solanum Brevi- dens* Using *Agrobacterium Tumefaciens*,” Plant Cell Reports 15:196-199 (1995), which is hereby incorporated by reference in its entirety). A total of thirty independent  
30 transgenic lines from *andigena* and twenty independent transgenic lines from ‘FL-1607’ were screened for insertion of the transgene and accumulation of *POTH1*

mRNA. Five independent transgenic lines of *S. tuberosum* spp. *andigena* and 4 lines of *S. tuberosum* cv. FL-1607 that showed high levels of *POTH1* mRNA accumulation were selected for further analysis. Untransformed tissue culture plants were used as controls.

5

#### **Example 6 -- Nucleic Acid Hybridizations**

[0126] Genomic DNA was isolated using the cetyltrimethylammonium bromide (CTAB) mini-plant DNA extraction method (Doyle et al., "A Rapid DNA Isolation Procedure for Small Quantities of Fresh Leaf Tissue," Phytochem. Bull. 19:11-15 (1987), which is hereby incorporated by reference in its entirety). DNA (10µg) was digested with *Hind* III or *Xba* I (Promega, Madison, WI), and gel electrophoresis was performed. DNA was denatured and blotted according to the methods described by Kolomiets et al., "A Leaf Lipxygenase of Potato Induced Specifically by Pathogen Infection," Plant Physiol. 124:1121-1130 (2000), which is hereby incorporated by reference in its entirety. Total RNA was isolated with TriPure Isolation Reagent (Roche Biochemicals, Indianapolis, IN) and gel electrophoresis was performed using 10 mM methyl mercury (II) hydroxide as a denaturant. For hybridization with *STGA20ox1*, shoot tip samples were collected at the same time of day to avoid variations due to diurnal regulation. Probes were labeled with [ $\alpha$ -<sup>32</sup>P]dCTP (RadPrime DNA Labeling System, Gibco BRL, Gaithersburg, MD). *POTH1* probes were generated by using the 730 nt *EcoR* I fragment of *POTH1* from the pCR2.1 vector (Invitrogen, Carlsbad, CA) with the ELK and homeodomains deleted. The 1.5 kb *EcoR* I -*Xho* I fragment of *StGA20ox1* cDNA (Carrera et al., "Feedback Control and Diurnal Regulation of Gibberellin 20-oxidase Transcript Levels in Potato," Plant Physiol. 119:765-773 (1999), which is hereby incorporated by reference in its entirety) was provided by Salomé Prat (Barcelona, Spain). All membranes were hybridized at 42 °C for 70 hours in 50% formamide. The membranes were rinsed in 2X SSC/0.1% SDS, at 25 °C, followed by 1X SSC /0.1% SDS for 0-20 minutes at 65 °C, then 0.1X SSC /0.1% SDS for 20-30 minutes at 65 °C. Film was exposed for 4 to 7 days.

### **Example 7 – Light Microscopy**

[0127] Leaf tissue was fixed in 2% glutaraldehyde, 2% paraformaldehyde in 0.1M sodium phosphate buffer pH 7.0 at 4 °C for 72 hours, dehydrated in a  
5 graded ethanol series, and embedded in LR White resin (Electron Microscopy Sciences, Ft. Washington, PA). One  $\mu\text{m}$  thick sections were cut on an ultramicrotome (Reichert/ Leica, Deerfield, IL) and stained with 1% toluidine blue. Sections were viewed and photodocumented using bright field microscopy.

### **Example 8 – GA Analysis**

[0128] Three replicates of shoot tips down to the sixth expanded leaf (10 g each), were harvested in liquid nitrogen and frozen at -80 °C. The tissue was ground with 80% methanol (MeOH) and incubated at 4 °C overnight. [ $^2\text{H}_2$ ]-GA internal standards were added in the following amounts in ng/g fwt: GA<sub>1</sub>: 1, GA<sub>8</sub>:  
15 10, GA<sub>19</sub>: 10, GA<sub>20</sub>: 20, and GA<sub>53</sub>: 5. The extract was filtered through Highflo Supercel and washed with 80% MeOH. After evaporation of the MeOH *in vacuo*, 0.5 M Na<sub>2</sub>HPO<sub>4</sub> was added to bring the pH to about 8.5, followed by addition of 20 mL of hexane. The flask was mixed well and the hexanes were evaporated off  
20 *in vacuo*. The solution was then acidified to pH 3-3.5 with glacial CH<sub>3</sub>COOH (acetic acid) and incubated for 15 minutes. The sample was then filtered through polyvinylpolypyrrolidone (PVPP) and washed with 0.2% acetic acid. The eluate was loaded onto a prepared Baker SPE (C<sub>18</sub>) cartridge and washed with 0.2% acetic acid. The sample was eluted off the column with 7 mL of 80% MeOH, evaporated to dryness, and dissolved in 1 mL 100% MeOH. The MeOH-insoluble  
25 precipitate was removed by centrifugation and the supernatant was evaporated to dryness, redissolved in 0.8 mL 0.2% acetic acid, and filtered through a 45  $\mu\text{m}$  filter. A one mL loop was used to load the sample onto the C<sub>18</sub> HPLC column (Econosphere: Phenomenex, Torrance, CA) run with the following 0.2% acetic acid to acetonitrile gradient: 5%-20% over 2 minutes; 20-35% over 15 minutes;  
30 35-75% over 15 minutes. Fractions for the following GAs were taken as follows: 10 - 14.3 minutes for GA<sub>8</sub>; 15.3 - 17.45 minutes for GA<sub>1</sub>; 23 - 27 minutes for

GA<sub>19</sub> and GA<sub>20</sub>; 27 - 29.3 minutes for GA<sub>53</sub>. Fractions were collected separately and methylated with diazomethane in ether. Samples were dried, redissolved in 1 mL ethyl acetate, and partitioned against water. The aqueous phase was partitioned against another 1 mL of ethyl acetate and the ethyl acetate fractions were combined. The samples were dried and placed under high vacuum over P<sub>2</sub>O<sub>5</sub>. The samples were dissolved in 2 µL dry pyridine and 10 µL BSTFA [bis(trimethylsilyl)trifluoro-acetamide] with 1 % TMCS (trimethylchlorosilane) (Sylon BFT: Pierce, Rockford, IL) and heated at 80 °C for 20 minutes. Samples were analyzed by GC-SIM on a GC-MS (HewlettPackard 5890 GC + 5970B MS) with a 15m Zebron ZB1 column (Phenomenex, Torrance, CA). The carrier gas, He, was set at a flow rate of approximately 35 cm/sec. The initial column temperature was 60 °C for one minute and then increased at a rate of 30 °C/minute to 240 °C, and then to 290 °C at a rate of 4 °C/minute. The injector temperature was 225 °C and the temperature of the detector was 300 °C. Concentrations of GA<sub>53</sub>, GA<sub>19</sub>, GA<sub>20</sub>, GA<sub>1</sub>, and GA<sub>8</sub> were determined by calculating the area of the peaks, 448/450, 434/436, 418/420, 506/508, and 594/596, respectively, at the correct Kovats retention indices. Reference spectra were obtained from Gaskin et al., "GC-MS of the Gibberellins and Related Compounds: Methodology and a Library of Spectra," Bristol UK: Cantock's Enterprises (1991), which is hereby incorporated by reference in its entirety. Cross-ion corrections were calculated according to the following formula where: R<sub>1</sub> = % endogenous ion in final; R<sub>2</sub> = % heavy ion in final; A<sub>1</sub> = % endogenous ion in natural unlabelled sample; A<sub>2</sub> = % heavy ion in natural unlabelled sample; B = heavy isotope internal standard.

$$\text{Amount of natural compound (A)} = \frac{[R_1]}{[R_2 \times A_1 - R_1 \times A_2]} \times \text{Amount of B added}$$

#### **Example 9 -- In vitro Tuberization**

[0129] Cuttings of transgenic and control plants were placed in Murashige-Skoog (MS) media plus 6% sucrose (Konstantinova et al., "Photoperiodic Control of Tuber Formation in Potato *Solanum Tuberosum* ssp. *Andigena* in vivo and in vitro," Russian J. Plant Physiol. 46:763-766 (1999), which is hereby incorporated by reference in its entirety). After 2 weeks under

long days (16 hours of light, 8 hours of dark) to promote rooting, plants were cultured separately under either long or short day (8 hours of light, 16 hours of dark) conditions. Plants were examined for tuber activity (percentage of plants that produced either swollen stolons or tubers) and the number of tubers were  
5 counted.

**Example 10 – Results: Isolation and Characterization of POTH1**

[0130] An early stage tuber cDNA library (Kang et al., “Nucleotide Sequences of Novel Potato (*Solanum tuberosum* L.) *MADS-box* cDNAs and Their  
10 Expression in Vegetative Organs,” Gene 166:329-330 (1995), which is hereby incorporated by reference in its entirety) from *Solanum tuberosum* ‘Superior’ was screened for members of the homeobox gene family. PCR primers were designed from the consensus sequence of the homeoboxes of the class I genes *kn1* from maize (Vollbrecht et al., “The Developmental Gene *Knotted-1* is a Member of a  
15 Maize Homeobox Gene Family,” Nature 350:241-243 (1991), which is hereby incorporated by reference in its entirety), *KNAT1* and *KNAT2* from Arabidopsis (Lincoln et al., “A *Knotted1*-like Homeobox Gene in Arabidopsis is Expressed in the Vegetative Meristem and Dramatically Alters Leaf Morphology When Overexpressed in Transgenic Plants,” Plant Cell 6:1859-1876 (1994), which is  
20 hereby incorporated by reference in its entirety), *OSHI* from rice (Matsuoka et al., “Expression of a Rice Homeobox Gene Causes Altered Morphology of Transgenic Plants,” Plant Cell 5:1039-1048 (1993), which is hereby incorporated by reference in its entirety), and *SBH1* from soybean (Ma et al., “Identification of a Homeobox-Containing Gene With Enhanced Expression During Soybean  
25 (*Glycine max* L.) Somatic Embryo Development,” Plant Mol. Biol. 24:465-473 (1994), which is hereby incorporated by reference in its entirety). A mass excision of the tuber cDNA library was performed, and this DNA was used as the PCR template. A band corresponding to the expected size of 158 nt was purified, cloned, and sequenced. This potato homeobox fragment was 87 % identical to the  
30 conserved positions of the consensus sequence created from the five class I genes, and was used as a probe to screen the cDNA library. Library screening resulted in the isolation of a truncated, 1053-nt homeobox cDNA from the library, which was

used as a probe to screen the library again. Three clones were isolated, and the full-length 1383-nt potato homeobox cDNA, *POTH1*, was selected for further study. The cDNA (Genbank Accession # U65648) includes an open reading frame of 1035 nt coding for a 345-residue protein estimated to have a mass of 37.95 kDa. It contains a 134-nt 5'-untranslated region, and a 216-nt 3'-untranslated region, including the poly-A tail. The coding sequence of the protein includes the 97-aa MEINOX domain, the 22-aa ELK domain, and the 64-aa homeodomain.

[0131] To identify proteins with similarity to *POTH1*, a BLAST analysis (Altschul et al., "Basic Local Alignment Search Tool," J. Mol. Biol. 215:403-410 (1990), which is hereby incorporated by reference in its entirety), was performed on the protein sequence and GAP analysis [Wisconsin Package Version 9.1, Genetics Computer Group (GCG), Madison, WI] was used to determine percent similarity. *POTH1* shares 86 % similarity with the homeodomain of KN1, classifying it as a class I homeobox protein (Kerstetter et al., "Sequence Analysis and Expression Patterns Divide the Maize *Knotted1*-like Homeobox Genes Into Two Classes," Plant Cell 6:1877-1887 (1994), which is hereby incorporated by reference in its entirety). However, over the entire protein sequence, *POTH1* shares only 51 % similarity with KN1. The five proteins with the most similarity to *POTH1* include TKN3 from tomato (U76408), NTH22 of tobacco (Nishimura et al., "The Expression of Tobacco *Knotted1*-type Class 1 Homeobox Genes Correspond to Regions Predicted by the Cytohistological Zonation Model," Plant J. 18: 337-347 (1999), which is hereby incorporated by reference in its entirety), PKN2 of *Ipomoea nil* (AB016000), KNAT2 of Arabidopsis (Lincoln et al., "A *Knotted1*-like Homeobox Gene in Arabidopsis is Expressed in the Vegetative Meristem and Dramatically Alters Leaf Morphology When Overexpressed in Transgenic Plants," Plant Cell 6:1859-1876 (1994), which is hereby incorporated by reference in its entirety) and NTH15 of tobacco (Tamaoki et al., "Ectopic Expression of a Tobacco Homeobox Gene, *NTH15*, Dramatically Alters Leaf Morphology and Hormone Levels in Transgenic Tobacco," Plant Cell Physiol. 38:917-927 (1997), which is hereby incorporated by reference in its entirety) with 94, 88, 73, 69, and 56 % similarity overall, respectively. As expected, relatively



high levels of conservation were observed in the homeodomains (97 to 83 %) and in the MEINOX domains (95 to 63 %) of this group.

**Example 11 – Results: Southern Analysis**

- 5    [0132]        To study the complexity of the *POTH1* gene family in the tetraploid potato genome, Southern analysis was performed. Genomic DNA from both *S. tuberosum* cv. FL-1607 and spp. *andigena* was digested with *Hind* III and *Xba* I. For both species, only two bands hybridized to a gene-specific probe for *POTH1* (Figure 1), indicating that *POTH1* is a member of a small gene family. A  
10   *Hind* III site is located within the cDNA sequence of *POTH1*.

**Example 12 – Results: Accumulation of *POTH1* mRNA**

- [0133]        Northern blot analysis was used to determine the pattern of *POTH1* mRNA accumulation in various organs of potato (Figure 2). Poly(A)+ enriched  
15   RNA samples were hybridized with a digoxigenin-UTP labeled 780-nt RNA antisense probe with the conserved ELK region, homeobox region, and poly-A tail deleted. A single band, approximately 1.3 kb in length, representing *POTH1* mRNA, was present in RNA extracted from stem, root, inflorescence, shoot apex, and swollen stolon apex (Figure 2, lanes 2, 3, 4, 6, and 7, respectively). *POTH1*  
20   transcripts were not detected in either mature leaf or mature tuber RNA (Figure 2, lanes 1 and 5). Equal loading and the quality of the RNA loaded were ascertained via ethidium bromide staining. This autoradiograph was representative of several replicate hybridization blots.

- [0134]        To determine more precisely the location of *POTH1* mRNA  
25   accumulation, *in situ* hybridization was performed on vegetative meristems of potato (Figure 3). The potato SAM is comprised of two tunica layers, which divide anticlinally to produce the epidermis and contribute to lateral organs such as leaves, and three corpus layers, which divide both periclinally and anticlinally to contribute to lateral organ and stem development (Esau, "The Stem: Primary  
30   State of Growth. In Wiley, eds., Anatomy of Seed Plants, 2nd Edition New York: pp. 243-294 (1977); Sussex, "Morphogenesis in *Solanum Tuberosum* L.: Apical

Structure and Developmental Pattern of the Juvenile Shoot,” Phytomorphology 5:253-273 (1955), which are hereby incorporated by reference in their entirety).

*POTH1* mRNA accumulates in the two tunica and three corpus layers of the SAM, the leaf primordia, the procambium, and the lamina of young leaves (Figure 3A).

- 5 Lower levels of *POTH1* transcript can also be detected in the developing leaflets of an older leaf (Figure 3A, OL). A slightly lower level of *POTH1* transcript can be detected in the central zone of the SAM, where initials divide less rapidly than adjacent cells.

- [0135] Potato plants produce underground stems that grow horizontally, called stolons (Jackson, “Multiple Signaling Pathways Control Tuber Induction in Potato,” Plant Physiol. 119:1-8 (1999), which is hereby incorporated by reference in its entirety). Under optimum conditions, the subapical region of the stolon tip will begin to swell and eventually develop into a tuber. A nontuberizing stolon will elongate with most of its growth occurring in the tunica and corpus layers.

- 15 The greatest concentration of *POTH1* signal can be detected in the apical meristem of the elongating stolon (Figure 3B). Expression levels are also high in the lamina of the youngest leaf, the procambium, and the perimedullary parenchyma associated with the vascular tissue (Figure 3B). Differentiation of the procambium into mature vascular tissue is marked by the appearance of xylem elements (Esau, “The Stem: Primary State of Growth. In Wiley, eds., Anatomy of  
20 Seed Plants, 2nd Edition New York: pp. 243-294 (1977), which is hereby incorporated by reference in its entirety), and *POTH1* transcript accumulates in this differentiated tissue as well (Figure 3B). No signal is detected in an elongating stolon tip hybridized with a sense *POTH1* probe (Figure 3C).

- 25 [0136] The apex of a tuberizing stolon, visibly swollen in Figure 3D, continues to accumulate *POTH1* mRNA in the apical meristem, the procambium, the lamina of new leaves, and the perimedullary parenchyma, but the signal is less intense than in the elongating stolon apical meristem (Figure 3B). In the subapical portion of the swollen stolon tip (Figure 3E), where rapid radial expansion is  
30 occurring (Xu et al., “Cell Division and Cell Enlargement During Potato Tuber Formation,” J Exp. Bot. 49:573-582 (1998), which is hereby incorporated by reference in its entirety), *POTH1* signal is detected, especially in the

perimedullary parenchyma, associated with the vascular tissue. There is some signal as well in the pith and inner cortex (Figure 3E). Figure 3F is the sense probe control corresponding to the section in Figure 3E. Similar results were observed with sense probe controls in each section examined. The data presented in Figure 3 is representative of several independent replications. Because Figures 3A-D are longitudinal sections through various apices at the same magnification, the location of labeled tissues is similar from one apex to the next.

**Example 13 -- Results: The Overexpression of *POTH1* in Transgenic Potato Plants**

[0137] To determine the effect of *POTH1* overexpression on the development of potato, the full-length *POTH1* sequence was placed under the control of the CaMV 35S promoter in the binary vector, pCB201 (Xiang et al., "A Mini Binary Vector Series for Plant Transformation," Plant Mol. Biol. 40:711-718 (1999), which is hereby incorporated by reference in its entirety). To examine the role of *POTH1* in tuberization, two cultivars of potato (*Solanum tuberosum* cv. FL-1607 and *S. tuberosum* ssp. *andigena*) were selected for transformation. *Andigena* plants are photoperiod sensitive, tuberizing only under short-day conditions (Carrera et al., "Changes in GA 20-oxidase Gene Expression Strongly Affect Stem Length, Tuber Induction and Tuber Yield of Potato Plants," Plant J. 22:1-10 (2000), which is hereby incorporated by reference in its entirety), whereas 'FL-1607' plants tuberize under both long- and short-day photoperiods. A total of thirty independent transgenic lines from *andigena* and twenty independent transgenic lines from 'FL-1607' were generated and screened for increased *POTH1* mRNA expression. Among 10 sense lines of *andigena* and 15 lines of 'FL-1607' that showed high levels of *POTH1* mRNA accumulation, five independent transgenic lines of *andigena* and 4 lines of 'FL-1607' were chosen for further analysis. An aberrant phenotype was observed only in those lines with detectable levels of *POTH1* mRNA from total RNA samples. Two transgenic lines, *andigena* lines 15 and 18 had the highest levels of *POTH1* mRNA accumulation (Figure 4A), whereas *andigena* lines 11, 20, and 29 had intermediate levels of *POTH1* mRNA (Figure 4A). Similar high levels of *POTH1*

accumulation were observed in 'FL-1607' overexpression lines that exhibited mutant phenotypes. Equivalent loading of RNA samples was verified by using an 18S rRNA probe from wheat (Figure 4B).

5    **Example 14 – Results: Phenotype of *POTH1* Overexpression Lines**

[0138]        Overexpression of *POTH1* resulted in a phenotype characterized by a reduction in plant height and leaf size (Figures 4C-F). Lines with the most abundant *POTH1* RNA levels had the greatest reduction in overall height. The height of potato subsp. *andigena* lines 15 and 18 was reduced by at least 64 % compared with wild-type plants (Figure 4C). Transgenic lines with an intermediate phenotype (*andigena* lines 20, 29, and 11) showed a 20 to 25 % reduction in plant height (Figure 4C). The decrease in plant height was due to a corresponding decrease in internode elongation (Figure 4D). The average internode length of the severe mutant, *andigena* line 15, was 4.0 mm compared to 16 mm for wild-type *andigena* plants. The same pattern was observed for petiole and leaflet length (Figure 4E and 4F) with the severe phenotypes displaying the greatest reduction in size. Among the five sense lines, petiole length was reduced by 70 to 96 %, whereas leaflet length was reduced by 29 to 87 % compared to wild-type. The sixth expanded leaf from the shoot apex was used to measure petiole and terminal leaflet length. Similar results were seen for 'FL-1607' overexpression lines.

[0139]        Transgenic plants that overexpressed *POTH1* also exhibited malformed leaves. The overall size of the leaflets was greatly reduced and they were rounded, curved, and wrinkled (Figure 5A-B). Wild-type leaflets have an ovate form and display pinnate venation with a prominent mid-vein (Figure 5B, left). In the overexpression mutants, the midvein is less prominent and the most severe phenotypes exhibited a 'mouse-ear' leaf phenotype (Figures 5B-D). The leaflets are heart-shaped with a shortened mid-vein. In addition, there has been a switch from pinnate to palmate venation (Figure 5B). The phyllotaxy is not altered in overexpression lines, although, compared with wild-type plants (Figure 5C), the leaves are clustered closer to the stem due to shortened petioles (Figure 5D). In tomato, the dominant mutations, *Mouse-ear* (*Me*) and *Curl* (*Cu*), were

- caused by a change in the spatial and temporal expression of the tomato *knox* gene *TKn2 /LeT6* (Parnis et al., "The Dominant Developmental Mutants of Tomato, *Mouse-ear* and *Curl*, are Associated With Distinct Modes of Abnormal Transcriptional Regulation of a *Knotted* Gene," Plant Cell 9:2143-2158 (1997);
- 5 Chen et al., "A Gene Fusion at a Homeobox Locus: Alterations in Leaf Shape and Implications for Morphological Evolution," Plant Cell 9:1289-1304 (1997), which are hereby incorporated by reference in their entirety). Overexpression of *kn1* (Hareven et al., "The Making of a Compound Leaf: Genetic Manipulation of Leaf Architecture in Tomato," Cell 84:735-744 (1996), which is hereby incorporated
- 10 by reference in its entirety) in tomato caused up to a six-fold increase in the level of leaf compoundness resulting in a leaf bearing 700-2000 leaflets. Such a marked increase in the level of compoundness was not observed in *POTH1* overexpression lines. Increased proliferation of leaflets from sense lines, however, was common (compare wild-type and line 19 leaflets in Figure 5E).
- 15 [0140] To determine whether *POTH1* overexpression affected the leaf at the cellular level, leaf cross-sections of the severe mutant, potato subsp. *andigena* line 15, were examined. Wild-type leaves consist of a palisade parenchyma layer on the adaxial side and a spongy parenchyma layer on the abaxial side (Figure 5F). The cells of the palisade layer are aligned in a vertical orientation and are
- 20 tightly packed, whereas the spongy parenchyma cells are more loosely arranged (Figure 5F). In leaves of potato subsp. *andigena* line 15, the palisade parenchyma layer is absent and the spongy parenchyma cells are more closely packed (Figure 5H). Overall cell size in the leaves of *andigena* line 15 is reduced by about one half.
- 25 [0141] Many of the traits of the phenotypes observed in *POTH1* overexpression lines were similar to GA-deficient mutants. These similarities included decreased plant height, decreased internode length, and darker green coloration of the leaves (van den Berg et al., "Morphology and [<sup>14</sup>C]Gibberellin A<sub>12</sub> Metabolism in Wild-Type and Dwarf *Solanum Tuberosum* ssp. *Andigena*
- 30 Grown Under Long and Short Photoperiods," J. Plant Physiol. 146:467-473 (1995), which is hereby incorporated by reference in its entirety). Because of this, exogenous GA<sub>3</sub> was applied to determine whether the overexpression lines were

responsive to GA treatment. The shoot apex of overexpression lines was sprayed to runoff with 10  $\mu$ M GA<sub>3</sub> in 0.002% (v/v) ethanol or with 0.002% (v/v) ethanol alone. Application of GA<sub>3</sub> not only caused plants with a severe phenotype to increase in height, but also partially rescued the leaf morphology of both severe  
5 and intermediate phenotypes. Palisade and spongy parenchyma organization is partially rescued in leaves from line 15 treated with GA<sub>3</sub> (Figure 5G). The compound leaf structure of the of the potato subsp. *andigena* wild-type leaf is shown in Figure 5I. The GA<sub>3</sub>-treated leaf (Figure 5J) of the severe mutant, line 15, is more similar in morphology to the wild-type leaf (Figure 5K). Leaflets are  
10 more ovate in form rather than the typical mouse-ear shape. Wild-type leaves have a prominent mid-vein (Figure 5L), whereas the mid-vein (Figure 5M, arrow) is more prominent in the mutant GA<sub>3</sub>-treated leaf than in the mutant untreated leaf (Figure 5N). The compound leaf structure of the ‘FL-1607’ wild-type leaf is shown in Figure 5O. The GA<sub>3</sub>-treated leaf (Figure 5P) of the severe mutant, ‘FL-  
15 1607’ line 5, is more similar in morphology to the wild-type leaf than to the mutant control leaf (Figure 5Q). Leaflets are more ovate in form rather than the typical ‘mouse-ear’ shape. The mid-vein (arrow) is more prominent in the GA<sub>3</sub>-treated leaf (Figure 5P) than in the mutant leaf (Figure 5Q).

[0142] To determine whether GA biosynthesis was disrupted in *POTH1*  
20 overexpression lines, levels of intermediates in the GA biosynthesis pathway in potato (van den Berg et al., “Metabolism of Gibberellin A12 and A12-aldehyde and the Identification of Endogenous Gibberellins in Potato (*Solanum tuberosum* ssp. *andigena*) Shoots,” *J. Plant Physiol.* 146:459-466 (1995), which is hereby incorporated by reference in its entirety) were measured. Levels of the  
25 intermediates GA<sub>53</sub> and GA<sub>19</sub> increased in *POTH1* overexpression lines, whereas GA<sub>1</sub> and GA<sub>8</sub> levels decreased (Figure 6). In potato subsp. *andigena* lines 29 and 20, GA<sub>53</sub> and GA<sub>19</sub> levels increased approximately 2-fold compared with wild-type lines (Figure 6). The levels of GA<sub>1</sub> and GA<sub>8</sub> present in potato subsp. *andigena* overexpression lines were approximately one-half that of wild-type  
30 levels (Figure 6). Accumulation of GA<sub>53</sub> and GA<sub>19</sub> with a concomitant decrease in GA<sub>1</sub> and GA<sub>8</sub> indicates that the GA biosynthetic pathway is blocked at the oxidation of GA<sub>19</sub> to GA<sub>20</sub>, leading to a decrease in the levels of bioactive GA<sub>1</sub>.

Similar patterns of accumulation for GA intermediates were also observed for potato subsp. *andigena* sense line 15 (in *andigena* line 15, GA<sub>53</sub> and GA<sub>19</sub> levels increased 4.8X and 2.1X, respectively, compared to wild-type).

[0143] Overexpression lines were deficient in bioactive GAs, but were responsive to the exogenous application of GA<sub>3</sub>. This indicates that GA biosynthesis is inhibited in the overexpression lines. In addition, accumulation of GA<sub>53</sub> and GA<sub>19</sub>, with a decrease in GA<sub>20</sub>, GA<sub>1</sub>, and GA<sub>8</sub> (Figure 6), indicates that the activity of the biosynthetic gene, GA 20-oxidase, may be suppressed. GA 20-oxidase catalyzes the oxidation of carbon 20 of GA<sub>53</sub> to GA<sub>44</sub> to GA<sub>19</sub> to GA<sub>20</sub>. The enzyme GA 3-oxidase then converts GA<sub>20</sub> to the active GA<sub>1</sub> (Hedden et al., "Gibberellin Biosynthesis: Enzymes, Genes and Their Regulation," Annu. Rev. Plant Physiol. Plant Mol. Biol. 48:431-460 (1997), which is hereby incorporated by reference in its entirety). To determine whether *POTH1* overexpression causes a change in GA 20-oxidase mRNA levels, RNA blot analysis was performed using one of the potato genes encoding GA 20-oxidase, *StGA20ox1*, as a probe (Carrera et al., "Feedback Control and Diurnal Regulation of Gibberellin 20-oxidase Transcript Levels in Potato," Plant Physiol. 119:765-773 (1999), which is hereby incorporated by reference in its entirety). In the overexpression lines, *StGA20ox1* mRNA levels were reduced substantially compared to levels in wild-type lines (Figure 7).

[0144] GA is involved in regulating cell growth in a tuberizing stolon (Xu et al., "The Role of Gibberellin, Absciscic Acid, and Sucrose in the Regulation of Potato Tuber Formation *in vitro*," Plant Physiol. 117:575-584 (1998), which is hereby incorporated by reference in its entirety) and in contributing to the control of the photoperiodic response of tuber formation (Martinez-Garcia et al., "The Interaction of Gibberellins and Photoperiod in the Control of Potato Tuberization," J. Plant Growth Regul. 20:377-386 (2001), which is hereby incorporated by reference in its entirety). Because levels of active GAs were reduced in transgenic plants, an *in vitro* tuberization assay (Konstantinova et al., "Photoperiodic Control of Tuber Formation in Potato *Solanum Tuberosum* ssp. *Andigena* *in vivo* and *in vitro*," Russian J. Plant Physiol. 46:763-766 (1999), which is hereby incorporated by reference in its entirety) was used to determine

the effect of *POTH1* overexpression on tuberization. After 2 weeks under a 16 hour light/8 hour dark photoperiod to induce rooting, plants were cultured on 6% (w/v) sucrose under either an 8 hour light/16 hour dark (inductive) or 16 hour light/8 hour dark (noninductive) photoperiod. After 10 days, the overexpression lines had 60 to 82% and 19 to 68% tuber activity under short and long days, respectively, compared to 0% activity for wild-type plants (Table 1).

**Table 1. *In vitro* tuberization of *POTH1* overexpression lines. *S. tuberosum* spp. *andigena* transgenics were placed on Murashige-Skoog media supplemented with 6% sucrose under either short-day (SD) or long-day (LD) conditions. At least 12 plants per line were monitored for total number of tubers that formed and tuber activity (percentage of plants that produced either swollen stolons or tubers). Numbers in parentheses are the average number of tubers produced per plant.**

# tubers (tubers/plant)			% tuber activity		
line	14d SD	14d LD	line	10d SD	10d LD
control	1 (.08)	1 (.06)	control	0	0
1200-29	21(1.4)	14 (.88)	1200-29	60	40
1200-11	13 (.72)	22 (1.2)	1200-11	78	68
1200-15	17 (1.5)	2 (.12)	1200-15	82	19
1200-18	12 (.86)	8 (.57)	1200-18	79	43
line	21d SD	21d LD			
control	(0.66)	(0.43)			
1200-29	(1.70)	(1.25)			
1200-11	(0.88)	(1.30)			
1200-15	(2.30)	(0.38)			
1200-18	(1.50)	(0.86)			

Tuber activity was calculated as the percentage of plants that formed either a swollen stolon or a tuber. At 14 days, overexpression lines produced an average of 0.7 to 1.5 tubers per plant under short days, whereas wild-type plants produced an average of 0.08 tubers per plant (Table 1). Similar results were observed under long days and after 21 days in culture (Table 1). Overall, the *POTH1* overexpression lines could produce more tubers in less time than controls and apparently, also overcome the negative effects of a long-day photoperiod on tuber



formation. The potato cv FL-1607 overexpression lines also exhibited increased tuber activity under both photoperiods.

5     **Example 15 – Discussion: *POTH1* Has a Widespread mRNA Expression Pattern**

- [0145]         Isolated from an early stage tuber cDNA library, *POTH1* is a homeobox gene belonging to the *knox* gene family. It contains the conserved homeodomain, ELK, and MEINOX domains. The homeodomain contains the invariant residues, PYP, between helices 1 and 2, making it a member of the
- 10    TALE superclass. Because of its close sequence match with the KN1 homeodomain, *POTH1* is classified as a *knox* class I homeobox gene.
- [0146]         Even though *POTH1* is classified as a class I *knox* gene, it has a more widespread mRNA expression pattern than other class I genes. *POTH1* is expressed in actively growing organs, but not in mature leaves or tubers. Unlike
- 15    the mRNA expression pattern of *kn1* which is limited to corpus cells of the apical meristem (Jackson et al., “Expression of Maize *KNOTTED1* Related Homeobox Genes in the Shoot Apical Meristem Predicts Patterns of Morphogenesis in the Vegetative Shoot,” Development 120:405-413 (1994), which is hereby incorporated by reference in its entirety), *in situ* hybridization showed that *POTH1*
- 20    mRNA accumulates in the meristematic and indeterminate cells of the SAM, determinate leaf primordia, the expanding lamina of new leaves, and developing leaflets of older leaves. The expression pattern of *POTH1* mRNA in the unswollen stolon is similar to that seen in the shoot apical meristem. Signal was highest in undetermined, meristematic cells, but was also detected in the lamina of
- 25    young leaves and the vascular tissue of the stem. Once tuberization has been initiated, the signal becomes less intense at the stolon apex, but is present in the vascular tissue in the subapical portion of the stolon. At this stage of tuberization, elongation of the meristem has stopped, and rapid, radial expansion occurs in the subapical region (Reeve et al., “Anatomy and Compositional Variation Within
- 30    Potatoes I. Developmental Histology of the Tuber,” Amer. Pot. J. 46:361-373 (1969), which is hereby incorporated by reference in its entirety).

[0147] Most class I *knox* genes have a more limited pattern of mRNA expression, restricted to undifferentiated cells of the meristem (Reiser et al., "Knots in the Family Tree: Evolutionary Relationships and Functions of *Knox* Homeobox Genes," Plant Mol. Biol. 42:151-166 (2000), which is hereby  
5 incorporated by reference in its entirety). Members of the tobacco *knox* family have distinct expression patterns within the SAM. *NTH15* and *NTH1* are expressed throughout the corpus, *NTH20* is expressed in the periphery zone, and *NTH9* is expressed in the rib zone of the SAM (Nishimura et al., "The Expression of Tobacco *Knotted1*-type Class1 Homeobox Genes Correspond to Regions  
10 Predicted by the Cytohistological Zonation Model," Plant J. 18: 337-347 (1999), which is hereby incorporated by reference in its entirety). The tomato *knox* class I genes, *TKn1* and *TKn2/LeT6*, have a expression pattern similar to *POTH1* with transcripts detectable in meristematic and differentiated cells. Expression of *TKn2/LeT6* was detected in the corpus of the meristem, developing leaf primordia,  
15 leaflet primordia and margins, and the vascular cells of the leaf (Chen et al., "A Gene Fusion at a Homeobox Locus: Alterations in Leaf Shape and Implications for Morphological Evolution," Plant Cell 9:1289-1304 (1997); Janssen et al., "Overexpression of a Homeobox Gene, *LeT6*, Reveals Indeterminate Features in the Tomato Compound Leaf," Plant Physiol. 117: 771-786 (1998), which are  
20 hereby incorporated by reference in their entirety). This expanded expression pattern in tomato has been attributed to the differences in compound leaf development compared to simple leaf development and the expansion of undifferentiated tissues to include developing leaflets. Potato is unique because it forms compound leaves from the vegetative shoot apical meristem above ground,  
25 but forms simple, scale leaves from the stolon meristem below ground (Sussex, "Morphogenesis in *Solanum Tuberosum* L.: Apical Structure and Developmental Pattern of the Juvenile Shoot," Phytomorphology 5:253-273 (1955), which is hereby incorporated by reference in its entirety). Expression of *POTH1* is detected in young leaves that arise from both the shoot apical and stolon  
30 meristems. This indicates that *POTH1* mRNA expression alone is not the determining factor for the development of compound leaves in potato. In the shoot or stolon meristem, the activity of *POTH1* may be regulated differently

through interaction with partner proteins specific for shoot or stolon meristem development.

**Example 16 – Discussion: Phenotype of *POTH1* Overexpression Transgenic Lines**

- 5 [0148] Overexpression of *POTH1* resulted in altered leaf morphology, dwarfism, and increased rates of *in vitro* tuberization. Leaves were small, wrinkled, and curved. Both severe and intermediate phenotypes were characterized by a ‘mouse-ear’ leaf phenotype. Leaves were heart-shaped with a
- 10 decreased midvein and palmate venation. The petioles were reduced in length resulting in leaves clustering closer to the stems. Overexpression lines exhibited dwarfism as a result of reduced internode length. The severity of the phenotype was correlated with the greatest levels of *POTH1* sense transcript accumulation. Cross-sections of leaves revealed that the mesophyll cell organization was
- 15 disrupted with the palisade parenchyma layer missing in *POTH1* overexpression lines. The tightly packed cells were about half the size of the wild-type cells. A similar disruption in leaf parenchyma cell layers was observed in sense mutants of *KNAT1* and *KNAT2* (Chuck et al., “*KNAT1* Induces Lobed Leaves With Ectopic Meristems When Overexpressed in Arabidopsis,” Plant Cell 8:1277-1289 (1996);
- 20 Frugis et al., “Overexpression of *KNAT1* in Lettuce Shifts Leaf Determinate Growth to a Shoot-like Indeterminate Growth Associated With an Accumulation of Isopentenyl-type Cytokinins,” Plant Physiol. 126:1370-1380 (2001); Pautot et al., “*KNAT2*: Evidence for a Link Between *Knotted*-like Genes and Carpel Development,” Plant Cell 13:1719-1734 (2001), which are hereby incorporated by
- 25 reference in their entirety). Because class I *knox* genes are implicated in maintaining the undifferentiated state of cells (Chan et al., “Homeoboxes in Plant Development,” Biochim. Biophys. Acta 1442:1-19 (1998), which is hereby incorporated by reference in its entirety), disruption in leaf architecture is likely a result of a defect in the normal differentiation program.
- 30 [0149] Based on overexpression phenotypes, *POTH1* and *NTH22* of tobacco (Nishimura et al., “Over-Expression of Tobacco *Knotted1*-type Class1 Homeobox Genes Alter Various Leaf Morphology,” Plant Cell Physiol. 41:583-

590 (2000), which is hereby incorporated by reference in its entirety) appear to have similar functions that overlap, but are distinct from, the class I *knox* genes, *kn1*, *NTH15*, *OSH1*, and *KNAT1*. Like overexpression of *POTH1* in potato and *NTH22* in tobacco, overexpression of *kn1*, *NTH15*, *OSH1*, *KNAT1* in tobacco or  
5 Arabidopsis (Sinha et al., “Overexpression of the Maize Homeo Box Gene, KNOTTED-1, Causes a Switch From Determinate to Indeterminate Cell Fates,” Genes Dev. 7:787-795 (1993); Sato et al., “Abnormal Cell Divisions in Leaf Primordia Caused by the Expression of the Rice Homeobox Gene OSH1 Lead to Altered Morphology of Leaves in Transgenic Tobacco,” Mol. Gen. Genet. 251:13-  
10 22 (1996); Tamaoki et al., “Ectopic Expression of a Tobacco Homeobox Gene, *NTH15*, Dramatically Alters Leaf Morphology and Hormone Levels in Transgenic Tobacco,” Plant Cell Physiol. 38:917-927 (1997); Chuck et al., “*KNAT1* Induces Lobed Leaves With Ectopic Meristems When Overexpressed in Arabidopsis,” Plant Cell 8:1277-1289 (1996); Lincoln et al., “A *Knotted1*-like Homeobox Gene  
15 in Arabidopsis is Expressed in the Vegetative Meristem and Dramatically Alters Leaf Morphology When Overexpressed in Transgenic Plants,” Plant Cell 6:1859-1876 (1994), which are hereby incorporated by reference in their entirety) resulted in dwarfism, decreased internode elongation, shortened petioles, and small deformed leaves. Additional phenotypes, including ectopic meristem formation,  
20 loss of apical dominance, and delayed senescence, however, were not observed in *POTH1* or *NTH22* overexpression transgenic lines. Whereas there seems to be some redundancy in function between different members of the *knox* gene family, (for example, regulation of GA biosynthesis), *POTH1* is not likely to have an identical function to *kn1*, *NTH15*, or *OSH1*. Rather, these genes are likely to have  
25 different subsets of target genes, which is reflected in their differences in homeodomain sequence (83 to 86 % match to *POTH1*’s homeodomain, compared to a 98 % match for *NTH22*).

30 **Example 17 – Discussion: Ectopic Expression of *POTH1* Results in GA Deficiency**

[0150] Similar to the *knox* genes *NTH15* of tobacco and *OSH1* of rice, the results above indicate that *POTH1* is a negative regulator of GA biosynthesis.

*POTH1* overexpression transgenic lines share many phenotypes with GA-deficient mutants including dwarfism, decreased internode elongation, and darker leaf coloration (van den Berg et al., "Morphology and [<sup>14</sup>C]Gibberellin A<sub>12</sub> Metabolism in Wild-Type and Dwarf *Solanum Tuberosum* ssp. *Andigena* Grown Under Long and Short Photoperiods," J. Plant Physiol. 146:467-473 (1995), which is hereby incorporated by reference in its entirety). Exogenous application of GA<sub>3</sub> partially rescued the aberrant leaf phenotype indicating that overexpression lines were responsive to GA. Levels of the bioactive GA, GA<sub>1</sub>, were reduced in overexpression lines, whereas intermediates prior to GA<sub>20</sub> in the pathway accumulated. Additionally, the mRNA levels of a key GA biosynthetic enzyme, GA 20-oxidase1, were reduced in overexpression lines. When *NTH15* and *OSHI* were overexpressed in tobacco, the levels of the hormones, auxin, cytokinin, abscisic acid, and GA were altered. GA<sub>1</sub> levels were reduced to 1.4% and 0.4-3.5% of controls in intermediate 35S-*NTH15* and severe or mild 35S-*OSHI* transgenics, respectively (Kusaba et al., "Alteration of Hormone Levels in Transgenic Tobacco Plants Overexpressing the Rice Homeobox Gene *OSHI*," Plant Physiol. 116:471-476 (1998); Tamaoki et al., "Ectopic Expression of a Tobacco Homeobox Gene, *NTH15*, Dramatically Alters Leaf Morphology and Hormone Levels in Transgenic Tobacco," Plant Cell Physiol. 38:917-927 (1997), which are hereby incorporated by reference in their entirety). In tobacco, *NTH15* affects plant growth by negatively regulating GA levels by suppressing the transcription of the tobacco GA 20-oxidase gene through a direct interaction with regulatory elements (Sakamoto et al., "KNOX Homeodomain Protein Directly Suppresses the Expression of a Gibberellin Biosynthetic Gene in the Tobacco Shoot Apical Meristem," Genes Dev. 15:581-590 (2001), which is hereby incorporated by reference in its entirety).

[0151] *POTH1* overexpression lines exhibited an increase in both the rate of tuberization and the total number of tubers formed under both short- and long-day photoperiods. These sense lines appear to have the capacity to overcome the negative effects of a long-day photoperiod on tuberization *in vitro*. Enhanced tuberization can be partially attributed to the decrease in GA<sub>1</sub> levels caused by *POTH1* suppression of GA 20-oxidase1. Phytochrome B (PHYB) and GAs are

involved in inhibiting tuberization under long-day photoperiods. A long-day photoperiod is sensed by the leaves and an inhibitory signal mediated by PHYB is transmitted from the leaves to the stolons to inhibit tuberization (Jackson, "Multiple Signaling Pathways Control Tuber Induction in Potato," Plant Physiol. 119:1-8 (1999), which is hereby incorporated by reference in its entirety). GA activity is regulated by light, decreasing under short-day photoperiods (Railton et al., "Effects of Daylength on Endogenous Gibberellins in Leaves of *Solanum Andigena* I. Changes in Levels of Free Acidic Gibberellin-like Substances," Physiol. Plant. 28:88-94 (1973), which is hereby incorporated by reference in its entirety) and is involved in the photoperiodic control of stolon growth. High levels of GA in the stolon tip favor elongation of stolon meristems, whereas decreasing levels are required for initiation of tuberization (Xu et al., "The Role of Gibberellin, Absciscic Acid, and Sucrose in the Regulation of Potato Tuber Formation in vitro," Plant Physiol. 117:575-584 (1998), which is hereby incorporated by reference in its entirety). GA 20-oxidase is a key enzyme in the GA biosynthetic pathway. In potato, the GA 20-oxidase genes are regulated by GA<sub>1</sub> feedback inhibition, blue light, and PHYB (Jackson et al., "Regulation of Transcript Levels of a Potato Gibberellin 20-Oxidase Gene by Light and Phytochrome B," Plant Physiol. 124:423-430 (2000), which is hereby incorporated by reference in its entirety). Whereas PHYB antisense plants were able to form tubers under both long- and short-day photoperiods (Jackson et al., "Phytochrome B Mediates the Photoperiodic Control of Tuber Formation in Potato," Plant J. 9:159-166 (1996), which is hereby incorporated by reference in its entirety), transgenic antisense *andigena* plants with suppressed levels of GA 20-oxidase1 (StGA20ox1) were not able to overcome the negative effects of photoperiod on tuberization in soil-grown plants (Carrera et al., "Changes in GA 20-oxidase Gene Expression Strongly Affect Stem Length, Tuber Induction and Tuber Yield of Potato Plants," Plant J. 22:1-10 (2000), which is hereby incorporated by reference in its entirety). While the experiments described above involved an *in vitro* assay rather than soil grown plants, Konstantinova et al., "Photoperiodic Control of Tuber Formation in Potato *Solanum Tuberosum* ssp. *Andigena* in vivo and in vitro," Russian J. Plant Physiol. 46:763-766 (1999), which is hereby incorporated by reference in its entirety, demonstrated that an *in*

*vitro* assay for tuber formation is a reliable method for ascertaining the effect of photoperiod on tuberization in a photoperiod responsive cultivar. While it is possible that GA levels are not reduced sufficiently in antisense GA 20-oxidase1 plants, an additional signal may be involved in the long-day-photoperiod inhibition of tuberization. This indicates that in addition to reducing GA levels, *POTH1* overexpression may enhance tuberization under long days by overcoming the effects of other negative regulators.

**Example 18 – Discussion: Regulation of *POTH1* Activity During Development**

[0152] Overexpression of *POTH1* potentially regulates development in the SAM and in underground stolons through a reduction in bioactive GA levels in vegetative meristems. Whereas GA levels are high in the elongating unswollen stolon and decrease in swollen stolons (Xu et al., “The Role of Gibberellin, Absciscic Acid, and Sucrose in the Regulation of Potato Tuber Formation in vitro,” Plant Physiol. 117:575-584 (1998), which is hereby incorporated by reference in its entirety), *POTH1* mRNA accumulates in both unswollen and swollen stolons. If *POTH1* is a negative regulator of GA synthesis, how can its expression mediate a decrease in GA levels in the swollen stolon leading to tuberization, but not in the elongating unswollen stolon tip? With other TFs, an interaction with a partner protein can regulate development by affecting the binding of the homeodomain(s) to the DNA of a target gene. In *Antirrhinum*, for example, formation of a ternary complex consisting of the MADS box proteins, SQUA, DEF, and GLO, greatly increases DNA binding compared to SQUA homodimers or DEF/GLO heterodimers alone (Egea-Cortines et al., “Ternary Complex Formation Between the MADS-box Proteins SQUAMOSA, DEFICIENS and GLOBOSA is Involved in the Control of Floral Architecture in *Antirrhinum majus*,” EMBO J. 18:5370-5379 (1999), which is hereby incorporated by reference in its entirety). The interaction of HOX proteins with PBC proteins in animals modulates the affinity of the HOX proteins for specific DNA binding sites (Chang et al., “Meis Proteins are Major in vivo DNA Binding Partners for Wild-Type but not Chimeric Pbx Proteins,” Mol. Cell. Biol. 17:5679-5687 (1997), which is hereby incorporated by

reference in its entirety). HOX homodimers have different DNA binding sites than HOX-PBC heterodimers (Mann et al., "Extra Specificity From *Extradenticle*: the Partnership Between HOX and PBX/EXD Homeodomain Proteins," Trends Genet. 12:258-262 (1996), which is hereby incorporated by reference in its  
5 entirety) indicating that the target gene (and function) is dependent on protein-protein interactions. Additionally, HOX-PBC complexes can be activators or repressors of transcription depending on the cell-type and the presence of a third interacting partner (Saleh et al., "Cell Signaling Switches HOX-PBX Complexes From Repressors to Activators of Transcription Mediated by Histone Deacetylases  
10 and Histone Acetyltransferases," Mol. Cell. Biol. 20:8623-8633 (2000), which is hereby incorporated by reference in its entirety). With the formation of different combinations of heterodimers and ternary complexes, the potential to regulate growth by interacting with different target genes is greatly increased.

[0153] It is clear that the interaction of KNOX proteins with other proteins  
15 is an important mechanism for regulating development. Protein-protein interactions between BEL-type TFs and KNOX proteins have been reported in barley (Müller et al., "*In vitro* Interactions Between Barley TALE Homeodomain Proteins Suggest a Role for Protein-protein Associations in the Regulation of *Knox* Gene Function," Plant J. 27:13-23 (2001), which is hereby incorporated by  
20 reference in its entirety) and Arabidopsis (Bellaoui et al., "The Arabidopsis BELL1 and KNOX TALE Homeodomain Proteins Interact Through a Domain Conserved Between Plants and Animals," Plant Cell 13:2455-2470 (2001), which is hereby incorporated by reference in its entirety). Homodimerization of KNOX proteins of barley (Müller et al., "*In vitro* Interactions Between Barley TALE  
25 Homeodomain Proteins Suggest a Role for Protein-protein Associations in the Regulation of *Knox* Gene Function," Plant J. 27:13-23 (2001), which is hereby incorporated by reference in its entirety) and rice (Nagasaki et al., "Functional Analysis of the Conserved Domains of a Rice KNOX Homeodomain Protein, OSH15," Plant Cell 13:2085-2098 (2001), which is hereby incorporated by  
30 reference in its entirety) has also been demonstrated. Sakamoto et al., "The Conserved KNOX Domain Mediates Specificity of Tobacco KNOTTED1-type Homeodomain Proteins," Plant Cell 11:1419-1431 (1999), which is hereby



incorporated by reference in its entirety, showed by expressing chimeric proteins in transgenic tobacco plants that the region of the MEINOX domain (designated KNOX2) involved in protein interaction was more important than the homeodomain in determining the severity of the mutant phenotype. By using a yeast two-hybrid library screen, as described in Examples 20-32, below, seven unique proteins were isolated from potato stolons that interact with POTH1. These seven proteins are homeobox genes of the BEL1-like family and members of the TALE superclass. Whereas *POTH1* has a widespread mRNA expression pattern, the seven potato BELs have a differential pattern of expression. It is possible that POTH1 interacts with one BEL protein to negatively regulate GA levels in the tuberizing stolon, but interacts with a different BEL partner in the elongating stolon or SAM. Overexpression of one of the POTH1-interacting proteins, StBEL-05, enhances tuberization under both long- and short-day photoperiods; but unlike POTH1 overexpression, leaf morphology is not altered (see below). In a tandem complex with a specific BEL partner, POTH1 could activate transcription of a set of target genes in one organ or set of cells and with another partner suppress those same genes in a different organ.

**Example 19 – Overexpression of POTH1 Negatively Regulates GA Levels and Affects Vegetative Morphology**

[0154] To further examine the function of POTH1, transformed potato plants (*Solanum tuberosum* spp. *andigena*) that overexpressed POTH1 mRNA were analyzed. For these experiments, the full-length cDNA sequence of POTH1 in a sense orientation driven by the CaMV-35S promoter in the binary vector, pCB201 (Xiang et al., “A Mini Binary Vector Series for Plant Transformation,” Plant Mol. Biol. 40:711-718 (1999), which is hereby incorporated by reference in its entirety) was used. The accumulation of the POTH1 mRNA was tightly correlated with a change in phenotype. These overexpressing lines were characterized by distorted, smaller leaves, and dwarfism (Figure 8). The mutant leaf traits are designated “mouse-ear” or “curled” phenotype as reported previously in other *knox* mutants (Parnis et al., “The Dominant Developmental Mutants of Tomato, *Mouse-Ear* and *Curl*, Are Associated with Distinct Modes of

Abnormal Transcriptional regulation of a *knotted* Gene," Plant Cell 9:2143-2158 (1997); Tamaoki et al., "Ectopic Expression of a Tobacco Homeobox Gene, *NTH15*, Dramatically Alters Leaf Morphology and Hormone Levels in transgenic Tobacco," Plant Cell Physiol. 38:917-927 (1997), which are hereby incorporated  
5 by reference in their entirety). Application of GA<sub>3</sub> produced a partial reversal of the leaf phenotype and completely rescued the dwarf phenotype (see above).

[0155] Because of the similarity of this POTH1 phenotype to those reported in tobacco (Tanaka-Ueguchi et al., "Overexpression of a Tobacco Homeobox Gene, *NTH15*, Decreases the Expression of a Gibberellin Biosynthetic  
10 Gene Encoding GA 20-oxidase," Plant J. 15:391-400 (1998); Tamaoki et al., "Transgenic Tobacco Over-Expressing a Homeobox Gene Shows a Developmental Interaction Between Leaf Morphogenesis and Phyllotaxy," Plant Cell Physiol. 40:657-557 (1999), which are hereby incorporated by reference in their entirety), the effect of GA 20-oxidase mRNA accumulation in these POTH1  
15 overexpressers was examined. GA 20-oxidase is a key biosynthetic enzyme in the GA pathway, catalyzing the conversion of GA<sub>53</sub> to GA<sub>20</sub> via GA<sub>44</sub> and GA<sub>19</sub> (Hedden et al., "Gibberellin Biosynthesis: Enzymes, Genes and Their Regulation," Ann. Rev. Plant Physiol. Plant Mol. Biol. 48:431-460 (1997), which is hereby incorporated by reference in its entirety). Using a probe for the potato  
20 GA 20-oxidase1 gene (Carrera et al., "Feedback Control and Diurnal Regulation of Gibberellin 20-oxidase Transcript Level in Potato," Plant Physiol. 119:765-773 (1999), which is hereby incorporated by reference in its entirety), a reduction in GA 20-oxidase1 mRNA in shoots of the most severe mutant phenotypes was observed (Figure 8). Both internode length and overall plant height were reduced  
25 approximately threefold in these mutant plants relative to controls. In addition, in a biochemical analysis performed in collaboration with Dr. Peter Davies, Cornell University, the levels of GA<sub>53</sub> and GA<sub>19</sub> increased, whereas the levels of GA<sub>20</sub> and GA<sub>1</sub> decreased in shoot tips of these plants. These results indicate that POTH1 is a negative regulator of GA biosynthesis and that it plays a role in controlling  
30 vegetative pattern formation.

**Example 20 – Two-Hybrid Selection and Deletion Analysis**

- [0156] The Matchmaker two-hybrid system (Clontech, CA) was used for the yeast two-hybrid screen. Yeast transformation and plasmid rescue into DH5- $\alpha$  *E. coli* cells were according to the manufacturer's instructions. Full-length
- 5 *POTH1* was cloned into the pBridge (Clontech, CA) vector and used as bait to screen the potato (*S. tuberosum* 'Desireé') stolon cDNA library in pAD-GAL4-2.1 (Stratagene, CA). Positive interactions were confirmed by cotransforming yeast strain AH109 with each purified pAD plasmid and pBridge: *POTH1* and plating on -leucine /-tryptophan (transformation control) and -leucine /-tryptophan /-
- 10 histidine/-adenine (selection) nutrient medium. Induction of the AH109 reporter gene, *lacZ*, was measured with a yeast  $\beta$ -galactosidase assay kit (Pierce Chemicals).  $\beta$ -galactosidase activity (Figure 9B) was determined from a known density of yeast cells and calculated as  $1000 \times OD_{420}/\text{time of color reaction (minutes)} \times \text{volume of yeast culture (ml)} \times OD_{600}$ .
- 15 [0157] The *StBEL-05* deletion constructs were amplified by PCR, then cloned into the vector, pGAD, in-frame with the GAL4 activation domain. *POTH1* deletion constructs were amplified by PCR, and cloned into pBridge (Clontech) in-frame with the GAL4 binding domain. Sequencing of selected cDNAs and constructs was performed at the Iowa State University DNA Facility.
- 20 For deletion analysis, modified constructs of *POTH1* were cloned into the pBridge vector for fusion with the DNA-binding domain of GAL4 (Figure 10A). For *StBEL-05*, constructs were cloned into the pGAD vector for fusion with the activating domain of GAL4 (Figure 10B). Deletion constructs were made from both the amino and carboxy termini. These mutants were then tested for
- 25 interaction in the yeast two-hybrid system by cotransforming into yeast strain AH109 with the corresponding full-length partner (*StBEL-05* in pGAL4 or *POTH1* in pBridge). All constructs were sequenced to verify that they were in-frame. Positive interactions were verified for *lacZ* induction by using a  $\beta$ -galactosidase assay (Pierce Chemical Company). For *POTH1*, seven deletion
- 30 constructs were tested (Figure 10A). For the BEL TFs, a fusion construct of

*StBEL-05* (653 aa of *StBEL-05* sequence) and nine deletion constructs were tested (Figure 10B).

- [0158] GenBank accession numbers for *StBEL-05*, -11, -13, -14, -22, -29, and -30 are AF406697, AF406698, AF406699, AF406700, AF406701, AF406702, AF406703, respectively.

#### **Example 21 -- *In vitro* Binding Assay**

- [0159] *In vitro* binding experiments were performed as described by Ni et al., "PIF3, a Phytochrome-Interacting Factor Necessary for Normal Photoinduced Signal Transduction, is a Novel Basic Helix-Loop-Helix Protein," *Cell* 95:657-667 (1998), which is hereby incorporated by reference in its entirety. The full-length sequence for *POTH1* was cloned into a pET17b/GAD fusion cassette and transcribed under the control of the T7 promoter. The BEL cDNAs were cloned into pGEM11Z vectors and were transcribed under the control of the T7 promoter. <sup>35</sup>S-methionine labeled bait and prey proteins were synthesized using the TnT *in vitro* transcription-translation kit (Promega) according to the manufacturer's protocols. Each 50 µl TnT reaction contained 2.0 µg of template plasmid DNA and 20 pmol (20µCi) of labeled <sup>35</sup>S-methionine. The POTH1:GAD/BEL complex was immunoprecipitated with anti-GAD antibodies (Santa Cruz Biotechnology, CA). The proteins from the pellet (one-half the fraction) and for the prey (one-fourth of the reaction volume) were resolved on a 10% SDS-PAGE gel and visualized by autoradiography.

#### **Example 22 -- Hybridization Blot Analysis**

- [0160] Total RNA was extracted from various organs of *Solanum tuberosum* ssp. *andigena* plants grown under a long-day photoperiod by using TRI REAGENT<sup>®</sup> according to the manufacturer's manual (Molecular Research Center, Inc., Cincinnati, OH). Swollen stolons (newly formed tubers) and tubers were harvested from short-day plants. For Figure 11B, RNA was extracted from leaves and stolons that were harvested from the photoperiod-responsive species

*Solanum tuberosum* ssp. *andigena* grown under a short-day photoperiod. Total RNA was size-fractionated via electrophoresis through a 1.4% agarose gel that contained 5.0 mM methyl-mercury hydroxide and transferred onto a MagnaGraph nylon membrane (Micron Separations Inc., Westboro, MA). Hybridization and washing conditions were the same as described by Kolomiets et al., “Lipoxygenase is Involved in the Control of Potato Tuber Development,” Plant Cell 13:613-626 (2001), which is hereby incorporated by reference in its entirety. For autoradiography, membranes were exposed to X-ray film with intensifying screens for three to six days at -80 °C. A 1.2 kb wheat 18S ribosomal RNA probe was used to confirm uniform loading of RNA for the blots in Figure 11A. Blots presented are representative examples of at least two independent experiments.

#### **Example 23 -- Plant Transformation**

[0161] Transformation and regeneration of plants was undertaken on leaf sections from *Solanum tuberosum* ssp. *andigena* line 7540 as described by Liu et al., “Transformation of *Solanum brevidens* Using *Agrobacterium tumefaciens*,” Plant Cell Reports 15:196-199 (1995), which is hereby incorporated by reference in its entirety. These autotetraploid *andigena* plants, strictly photoperiodic for tuberization, were obtained from the Institut für Pflanzenbau und Pflanzenzüchtung, Braunschweig, Germany. The sense constructs were made from a 2.0 kb fragment from the *StBEL-05* cDNA and cloned into the binary vector pCB201 (Xiang et al., “A Mini Binary Vector Series for Plant Transformation,” Plant Mol Biol 40:711-718 (1999), which is hereby incorporated by reference in its entirety) driven by the constitutive CaMV-35S promoter. Constructs were checked by using PCR with clone-specific primers. Positive recombinants were transferred to the *Agrobacterium tumefaciens* strain GV2260 by using the procedure of direct transformation (An et al., Binary vectors. in Plant Mol. Biol. Manual, pp. A3:1-19, Kluwer Academic, Belgium (1988), which is hereby incorporated by reference in its entirety). Control plants in the tuberization study were *andigena* plants regenerated *in vitro*. Functional transformants were identified by PCR analysis of genomic DNA and by detection of the accumulation of sense transcripts of *StBEL-05* in shoot tip samples. From among these

positives, the seven independent transformants (lines 7, 11, 12, 14, 16, 19, and 20 for *StBEL-05*) used in this study were selected on the basis of abundant accumulation of sense mRNA in shoot tips. Quantitative analysis of cytokinins was performed by using liquid chromatography as described above. Three  
5 replicate 200 mg (fresh wt) samples of shoot tips down to the fourth visible expanded leaf were collected, frozen in liquid nitrogen, lyophilized, and analyzed.

#### **Example 24 – Evaluation of Tuber Formation**

[0162] For *in vitro* tuberization, cultured transgenic plants were grown on  
10 a Murashige and Skoog medium with 6.0 % sucrose under a long-day photoperiod (16 hours of light, 8 hours of dark) in a growth chamber for two weeks and then transferred to a short-day photoperiod (8 hours of light, 16 hours of dark) in the same growth chamber. For tuber induction, plants were evaluated daily for tuber formation. Soil-grown plants were grown in 10-cm pots under long days (16  
15 hours of light, 8 hours of dark) in the greenhouse supplemented with high pressure sodium HID lamps until they reached the 16-leaf stage and then transferred to short days in the growth chamber. After 14 days under short days, plants were evaluated for tuber formation.

#### **Example 25 – Results: Isolation of Potato KNOX Interactive Proteins**

[0163] Making use of the two-hybrid selection system in yeast,  
approximately 10<sup>6</sup> transformants from a stolon cDNA library of potato were screened using *POTH1* in the GAL4-binding domain vector, pBridge (Clontech), as bait. Thirty-eight positive clones that grew on selective media were identified.  
25 Of the 38 that were sequenced, 23 clones could be grouped into seven unique genes encoding different members of the TALE superclass of transcription factors (Chan et al., “Homeoboxes in Plant Development,” Biochim Biophys Acta 1442:1-19 (1998), which is hereby incorporated by reference in its entirety). All seven, designated *StBEL-05*, *-11*, *-13*, *-14*, *-22*, *-29*, and *-30* (GenBank accession  
30 numbers AF406697, AF406698, AF406699, AF406700, AF406701, AF406702, AF406703, respectively) showed selective interaction when tested in the yeast

system both for nutritional markers and for *lacZ* activation (Figure 9A and 9B). Interaction occurred also when the prey cDNAs were cloned into pBridge and transformed with POTH1 in a GAL4-activation domain vector. As a test for autoactivation, the pAD transformants (5, 11, 13, 14, 22, 29, 30) did not grow on  
5 -histidine, -adenine, and -leucine medium and the pBD transformant did not grow on -histidine, -tryptophan, and -adenine medium. *In vitro* binding experiments verified the results of the two-hybrid selection. POTH1 pulled down three representative StBEL proteins with divergent sequence similarity in the BELL domain (5, 13, and 30) and synthesized by *in vitro* transcription/translation in  
10 immunoprecipitation assays (Figure 9C).

**Example 26 -- Results: The Proteins that Interact with the Potato KNOX protein are Members of the BEL Family of Transcription Factors**

15 [0164] A phylogenetic analysis of the sequences of the seven interacting proteins identified them as members of the BEL1-like family of transcription factors (Figure 12). These seven can be organized into four subgroups based on amino acid sequence similarity. Three clones (*StBEL-05*, -11, and -29) had 60-69% similarity to each other overall and two other clones had a 78% match  
20 (*StBEL-13* and -22). These two groups range in similarity to the others from 45-53% and a third (*StBEL-30*) has about 51% similarity to the others. The sequence similarity of *StBEL-14* to the other six ranged from 45 to 56%. The amino acid sequence of StBEL-05 has overall 56% similarity to BLH1 of *Arabidopsis* that interacts with KNAT1 (GenBank accession number AAK43836), *StBEL-13*  
25 matches an apple BEL (Dong et al., "MDH1: an Apple Homeobox Gene Belonging to the BEL1 Family," *Plant Mol Biol* 42:623-633 (2000), which is hereby incorporated by reference in its entirety, GenBank accession number AAF43095) at 74% similarity, and *StBEL-30* matches another *Arabidopsis* BEL (GenBank accession number T05281) at 59% similarity. The close match of all  
30 seven to the conserved homeodomain and the presence of the proline-tyrosine-proline (P-Y-P) loop between helices I and II (Figure 13A) distinguish these novel proteins as BEL types in the TALE superclass (Bürglin, "Analysis of TALE Superclass Homeobox Genes (MEIS, PBC, KNOX, Iroquois, TGIF) Reveals a

Novel Domain Conserved Between Plants and Animals," Nucleic Acids Res 25:4173-4180 (1997), which is hereby incorporated by reference in its entirety). The homeodomain region is nearly identical among these seven (Figure 13A, encompassing helices I, II, and III). Other regions of conserved sequence identity  
5 are shown schematically in Figure 13A. These include the amino-terminal SKY box consisting of 20 aa (from ser-207 to lys-226 in StBEL-05), the 120-aa domain starting at leu-272 of the StBEL-05 sequence, and the carboxy-terminal VSLTLGL-box (SEQ ID NO:15) beginning at val-620. Three  $\alpha$ -helices were predicted from the conserved 120-aa region of the BEL protein StBEL-05  
10 (underlined sequence of Figure 13B). Among the seven BELs, the percent similarity of the amino acid sequence in this conserved 120-aa domain ranged from 58 to 90%. Bellaoui et al., "The Arabidopsis BELL1 and KNOX TALE Homeodomain Proteins Interact Through a Domain Conserved Between Plants and Animals," Plant Cell 13:2455-2470 (2001), which is hereby incorporated by  
15 referenced in its entirety, referred to this region as the BELL domain.

[0165] The deduced lengths of the seven original cDNAs are 688 aa for *StBEL-05*, 535 aa for *StBEL-11*, 586 aa for *StBEL-13*, 589 aa for *StBEL-14*, 620 aa for *StBEL-22*, 567 aa for *StBEL-29*, and 645 aa for *StBEL-30*. Five'-RACE was used to verify the full-length of *StBEL-05*, -13, -14 and -30. For blot  
20 hybridizations, a representative clone from each of the four subgroups (*StBEL-05*, -13, -14, and -30) was used. Southern blot analysis revealed that these genes are unique and belong to small gene subfamilies, based on the complexity of bands detected by gene-specific probes from each of the cDNAs (Figure 13C).

## 25 **Example 27 -- Results: Patterns of mRNA Accumulation for the Potato BELs**

[0166] The BEL1-like gene represented by *StBEL-05* exhibited mRNA accumulation in all organs examined, with the greatest levels in leaves and stems (Figure 11A). Transcript accumulation of *StBEL-11* and *StBEL-29* was similar to the pattern of *StBEL-05*. Transcripts for *StBEL-13* accumulated to the highest  
30 levels in the SAM and in fully formed flowers but were barely detectable in other organs (Figure 11A). The autoradiographs for *StBEL-13* were exposed two-times



longer than the other StBELs. For *StBEL-14*, transcripts were detected in flowers, leaves, roots, and stolons. The greatest accumulation of *StBEL-30* was in flowers with detectable levels in all organs examined. To examine more closely the dynamics of StBEL expression during tuber induction, a temporal study was undertaken for the accumulation of *StBEL-05* transcripts in leaves and stolons of the photoperiod-sensitive potato species *S. tuberosum* ssp. *andigena* grown under short-day conditions. Steady-state levels of *StBEL-05* mRNA increased in both leaves and stolons after exposing the plants to short-day (SD) conditions (Figure 11B). Visible tuber formation for the plants grown under SD conditions was observed between 10 to 14 days. In this study, the accumulation of mRNA for the BEL cDNA, *StBEL-05*, was linked to the induction of tuber formation in the leaves and stolons of a potato species responsive to a SD photoperiod. In addition, a temporal study was undertaken for the accumulation of BEL transcripts in stolons of the photoperiod-sensitive potato species *S. demissum* grown under short-day conditions. The induction of *StBEL-05*, *StBEL-14*, and *StBEL-30* expression was first detected in stolons one day after exposing the plants to short-day (SD) conditions (Figure 11C). This increase in RNA levels remained steady through 7 days. Transcripts for *StBEL-13* were not detected in stolons in any stage of development (Figure 11C). Visible tuber formation for the plants grown under SD conditions was observed between 10 to 14 days. In this study, the accumulation of mRNA for the BEL cDNAs, *StBEL-05*, *StBEL-14*, and *StBEL-30* was linked to the induction of tuber formation in the stolons of a potato species responsive to a SD photoperiod.

**Example 28 – Results: Determining the Protein Binding Regions in POTH1 and the BEL-Like Proteins**

[0167] Interaction with StBEL-05 was observed with all deletions outside the KNOX domain, with pBHD2 (missing the amino-terminus and the first 48 aa of the KNOX domain, Figure 10A), with pBHD6 (missing the carboxy terminus and 52 aa of the carboxy-end of the KNOX domain), and with pBHD-9 (first amino-terminal 114 aa but no KNOX domain sequence). No interaction was observed with pBHD3 (missing all of the KNOX domain and the first 114 aa).

Control experiments identified the first 114 aa of the N-terminus (pBHD9) as a transcriptional activator. This construct transformed alone into AH109 exhibited nutrient selection on -histidine, -tryptophan, and -adenine medium. Co-transformation of pBHD9 with an empty pGAD cassette produced transformants  
5 capable of growth on - histidine, - tryptophan, - adenine, and -leucine medium and induction of *lacZ*. None of the other constructs containing this domain were capable of growing on selection media without StBEL-05. Using the *in vitro* binding protocol, both the pBHD6 construct, containing the amino-terminal half of the KNOX domain, and the pBHD9 construct were unable to pull-down  
10 StBEL-05. When the pBDH9 construct was cloned into the pGAD vector, no interaction was observed with StBEL-05 in pBridge.

[0168] Fusion constructs of *StBEL-05* that dissected the 120-aa domain (pAD5-2, -3, -4, -9, and -11) were tested because this is one of the regions that is conserved in BEL TFs from other plant species (Bellaoui et al., "The Arabidopsis  
15 BELL1 and KNOX TALE Homeodomain Proteins Interact Through a Domain Conserved Between Plants and Animals," Plant Cell 13:2455-2470 (2001), which is hereby incorporated by reference in its entirety; Figure 13B). Interaction with POTH1 was observed with all constructs that had deletions exclusively outside of the conserved 120-aa box (Figure 10B). The only exception to this was with  
20 pAD5-9 that demonstrated an interaction and included a 43-aa deletion from the carboxy end of the 120-aa domain. Even with as little as a 27-aa deletion from the amino end of the 120-aa domain, interaction did not occur (Figure 13B, Figure 10B, pAD5-2). Similar to the results of Bellaoui et al., "The Arabidopsis BELL1 and KNOX TALE Homeodomain Proteins Interact Through a Domain Conserved  
25 Between Plants and Animals," Plant Cell 13:2455-2470 (2001), which is hereby incorporated by reference in its entirety, deletion of the SKY box (construct pAD5-1) resulted in a 55 % decrease in the induction of the *lacZ* marker as measured by  $\beta$ -galactosidase activity relative to the full-length construct, StBEL-05 (Figure 10B).

30

**Example 29 – Results: Enhanced Tuber Formation in Transgenic Plants That Overexpress the BEL cDNA, StBEL-05**

[0169] To examine the function of the potato BELs, transformed potato plants (*Solanum tuberosum* ssp. *andigena*) that over expressed *StBEL-05* from a constitutive promoter were analyzed. This BEL gene was selected because of its moderate level of activity in stolons and tubers and its increase in RNA levels in response to inductive conditions for tuber formation (Figure 11). For these experiments, a 2000-bp fragment of the coding sequence of *StBEL-05* in a sense orientation driven by the CaMV-35S promoter in the binary vector pCB201 (Xiang et al., “A Mini Binary Vector Series for Plant Transformation,” Plant Mol Biol 40:711-718 (1999), which is hereby incorporated by reference in its entirety) was used. Transformants were identified by PCR analysis of genomic DNA and by detection of the accumulation of sense transcripts of *StBEL-05* in RNA samples from vegetative meristems. From among approximately twenty-five positives, four independent lines with the highest levels of *StBEL-05* mRNA accumulation (Figure 14A) were selected for evaluation of tuber formation *in vitro* under both inductive (SD) and noninductive (LD) conditions. The highest expressers of *StBEL-05* sense transcripts (lines 11, 12, 14, and 19) exhibited tuber formation under LD conditions (Figure 14B). Control plants (WT and line 6) produced tubers only under SD conditions. The highest overexpressers of *StBEL-05* also produced more tubers than control plants over the course of this experiment and were more responsive to inductive conditions. After seven days under SD conditions, the control plants had produced no tubers, whereas the overexpression mutants (lines 11, 12, 14, and 19) had produced 10, 8, 15, and 4 tubers, respectively (Figure 14B). After 14 days under SD, controls had increased to 6 and 4 tubers, whereas the overexpression lines had increased to 12, 14, 24, and 10 tubers, respectively. Tuber yields (fr wt) also increased in overexpression lines 12, 14, and 19 (Figure 14C). The greatest tuber production was exhibited by lines 12 and 14 with a five- and sixteenfold increase, respectively, relative to wild-type plants (Figure 14B, bottom panel). Tubers from the overexpression lines grew larger than controls. Select tubers from line 14 reached fresh weights of almost 700 mg, whereas the largest control tuber reached only 140 mg.

[0170] With whole plants grown in soil under SD conditions for 14 days, *StBEL-05* overexpression lines produced an average of three- to fivefold more tubers per plant and more than a threefold greater tuber yield per plant than controls (Table 2).

- 5 **Table 2. Rate of tuberization for overexpression lines of *StBEL-05* under soil-grown, short-day conditions. Plants were grown in 10-cm pots under long days (16 hours of light, 8 hours of dark) until they reached the 16-leaf stage and then transferred to short days (8 hours of light, 16 hours of dark). After**  
10 **14 days under short days, four plants per independent line were evaluated for tuber formation. Standard errors of the mean are shown.**

Plant line	Number tubers plant <sup>1</sup>	Tuber yield plant <sup>1</sup> (g)
Wild-type	2.2 ± 1.4	1.4 ± 0.9
StBEL5-12	8.0 ± 0.8	5.4 ± 1.3
StBEL5-14	8.3 ± 0.9	4.6 ± 1.3
StBEL5-19	11.5 ± 2.1	4.7 ± 1.4

- Increased yields (as high as 50%) were maintained for these lines even after six  
15 weeks of growth in soil. Seven overexpressing sense lines (lines 7, 11, 12, 14, 16,  
19, and 20) also exhibited tuber activity (swollen stolons or tuber formation) on  
soil-grown plants under LD greenhouse conditions. Five of these plants produced  
tubers, whereas control plants exhibited no tuber activity. In addition, the rate of  
tuberization for plants grown *in vitro* under short-day conditions for 21 days is  
20 shown in Table 3, below.

**Table 3. Rate of tuberization for overexpression lines of StBEL-05. Plants were grown *in vitro* under short days in media with 6% sucrose for 21 days and scored for tuber formation. Twenty-five plants per independent line were evaluated, thirty-five for controls.**

Plant line	Number tubers plant <sup>-1</sup>	Tuber yield plant <sup>-1</sup> (mg)
Control	0.4	18
StBEL-05-12	0.9	95
StBEL-05-14	1.3	292
StBEL-05-19	0.9	50

5

Similar to *POTH1* overexpressers (see above), these results show that the accumulation of *StBEL-05* mRNA is correlated with an increased rate of tuber formation. Other than this enhanced capacity for tuberization, the *StBEL-05* overexpression lines in Table 2 did not exhibit the phenotype characteristic of KNOX gene overexpressers, including extreme dwarfism and abnormal leaf morphology (Figure 15). The abnormal phenotype of KNOX overexpressers is mediated by changes in hormone levels, specifically, a reduction in gibberellins and an increase in cytokinins (see above; Sato et al., "Abnormal Cell Divisions in Leaf Primordia Caused by the Expression of the Rice Homeobox Gene OSH1 Lead to Altered Morphology of Leaves in Transgenic Tobacco," *Mol Gen Genet* 251:13-22 (1996); Tamaoki et al., "Ectopic Expression of a Tobacco Homeobox Gene, *NTH15*, Dramatically Alters Leaf Morphology and Hormone Levels in Transgenic Tobacco," *Plant Cell Physiol* 38:917-927 (1997); Frugis et al., "Overexpression of *KNAT1* in Lettuce Shifts Leaf Determinate Growth to a Shoot-like Indeterminate Growth Associated With an Accumulation of Isopentenyl-type Cytokinins," *Plant Physiol* 126:1370-1380 (2001), which are hereby incorporated by reference in their entirety). With the exception of two *StBEL-05* sense mutants (lines 11 and 20), the leaf and stem growth of the *StBEL-05* overexpression lines was similar to wild-type plants (Figure 15). All five

*StBEL-05* lines exhibited an enhanced rate of growth comparable to control plants (Table 4).

5 **Table 4. Plant height (cm) and fresh weight (g) of overexpression lines of *StBEL-05* under soil-grown, long-day conditions. Plants were grown in 10-cm pots under long days (16 hours of light, 8 hours of dark) and plant height was measured after 10 and 45 days. Four plants per independent line were evaluated for growth. Fresh weight of leaves and stems was measured after 45 days. Standard errors of the mean are shown.**

Plant Line	Plant height (cm)		Fresh weight (g) of stem and leaves
	at 10 d	at 45 d	
Wild type	5.3 ± 0.3	35.2 ± 2.2	18.0 ± 2.6
StBEL5-11	7.3 ± 0.4	31.9 ± 3.0	19.6 ± 1.3
StBEL5-20	6.3 ± 0.6	32.2 ± 2.0	10.8 ± 0.5
StBEL5-12	7.1 ± 0.7	44.9 ± 0.9	23.3 ± 1.2
StBEL5-14	6.2 ± 0.2	38.2 ± 1.2	29.2 ± 1.0
StBEL5-19	7.1 ± 0.5	48.7 ± 1.9	25.5 ± 3.5

- 10 The average height of line 19 plants was 13.5 cm greater than control plants after 45 days. Fresh weights of leaves and stems of lines 12, 14, and 19 were 29 to 62 % greater than control plants. Lines 11 and 20 exhibited a more rapid rate of growth early (10 days) and then growth rate dropped off by 45 days (Table 4). Accumulation of *StBEL-05* transgenic mRNA in line 20 was equivalent to line 11.
- 15 Three-month old plants from lines 11 and 20 exhibited a slight reduction in leaf size and stem height as a result of decreased apical dominance. To examine the mechanism for this reduced leaf morphology, cytokinin analysis was performed on shoot apices down to the fourth visible true leaf. Similar to *POTH1*

overexpressers, shoot tips of both *StBEL-05* lines 11 and 20 exhibited a two- to fivefold increase in the bioactive forms of cytokinin (Table 5).

5 **Table 5. Cytokinin content (picomoles g fr wt<sup>-1</sup>) in shoot tips of *POTH1* and *StBEL-05* overexpression lines grown under long days (16 hours of light, 8 hours of dark) in the greenhouse. Wild-type lines are nontransformed *Solanum tuberosum* spp. *andigena*. Zeatin types include zeatin, zeatin riboside, dihydrozeatin, and dihydrozeatin riboside. Isopentenyl types include isopentenyl and isopentenyladenine. Standard error was calculated on three replicates.**

10

Sample	Zeatin types	Isopentenyl types
Wild-type	10.5 ± 1.0	12.0 ± 1.5
POTH1-15	42.5 ± 15	35.5 ± 7.0
POTH1-29	34.0 ± 12	30.0 ± 6.0
StBEL5-11	55.5 ± 30	31.5 ± 11
StBEL5-20	30.5 ± 6.0	29.5 ± 6.5

The overall magnitude increases in the cytokinin types among the four *StBEL* and *POTH1* mutant lines were remarkably consistent.

15 [0171] *POTH1* sense lines had increased levels of GA<sub>53</sub> and GA<sub>19</sub> and decreased levels of GA<sub>20</sub> and GA<sub>1</sub> in shoot tips, indicating a down-regulation of the biosynthetic enzyme GA 20-oxidase1 (see above). Using a probe for the potato GA 20-oxidase1 gene (Carrera et al., "Changes in GA 20-oxidase Genes Expression Strongly Affect Stem Length, Tuber Induction and Tuber Yield of Potato Plants," *Plant J.* 22:1-10 (2000), which is hereby incorporated by reference

20 in its entirety), a reduction in GA 20-oxidase1 mRNA in shoots of the most severe mutant phenotypes for *POTH1* sense lines was observed (see above, Figure 15).

To determine the effect of overexpression of the POTH1 partner, StBEL-05, RNA levels for GA 20-oxidase1 were examined in the stolons of *StBEL-05* sense lines grown under long-day photoperiod conditions. All three of the *StBEL-05* lines examined (lines 11, 12, and 20) exhibited a reduction in GA 20-oxidase1 mRNA in stolon tips comparable to controls (Figure 16). No such reduction in the levels of GA 20-oxidase1 mRNA was observed in shoot tips of StBEL-05 lines grown under long days.

[0172] To determine the effect of upregulating *StBEL-05* mRNA levels on *POTH1* RNA accumulation, northern blots were performed on total RNA extracted from *StBEL-05* sense lines 12, 14, 19, and 20 using *POTH1* as a probe. There were no changes in the levels of *POTH1* mRNA in both shoot tips and stolon tips of these *StBEL-05* lines relative to wild-type plants. These results indicate that the enhancement of tuber formation in *StBEL-05* overexpression lines is not mediated by an indirect increase in *POTH1* expression.

15

### **Example 30 – Discussion: Seven BEL Proteins Interact With a KNOX Protein of Potato**

[0173] Using a yeast two-hybrid library screen, seven unique proteins from potato stolons that interact with the *knotted*-like protein, POTH1, were identified. Sequence analysis revealed that these interacting proteins are from the BEL1-like family in the TALE superclass of homeodomain proteins. These proteins have conserved regions in common with other TALE proteins, including the homeodomain (comprised of three  $\alpha$ -helices) and the proline-tyrosine-proline "TALE" (Bürglin, "Analysis of TALE Superclass Homeobox Genes (MEIS, PBC, KNOX, Iroquois, TGIF) Reveals a Novel Domain Conserved Between Plants and Animals," Nucleic Acids Res 25:4173-4180 (1997), which is hereby incorporated by reference in its entirety). These sequences have been implicated in DNA-binding and protein/protein interactions, respectively (Mann et al., "Extra Specificity From *extradenticle*: the Partnership Between HOX and PBX/EXD Homeodomain Proteins," Trends in Genet 12:258-262 (1996); Passner et al., "Structure of DNA-Bound Ultrabithorax-Extradenticle Homeodomain Complex,"



Nature 397:714-719 (1999), which are hereby incorporated by reference in their entirety). A second conserved region of 120 aa just upstream from the homeodomain (designated the BELL domain by Bellaoui et al., "The Arabidopsis BELL1 and KNOX TALE Homeodomain Proteins Interact Through a Domain Conserved Between Plants and Animals," Plant Cell 13:2455-2470 (2001), which is hereby incorporated by reference in its entirety) was identified among BEL proteins by using a BLAST analysis (Figure 13B, bold letters). Sequence analysis of the predicted secondary structure of this domain reveals the presence of three putative  $\alpha$ -helices within the 120 residues (Figure 13B, underlined sequence).

Not all BEL proteins conserve the third helix, however, including the *Arabidopsis* BEL, ATH1 (Quaedvlieg et al., "The Homeobox Gene ATH1 of *Arabidopsis* is Depressed in the Photomorphogenic Mutants cop1 and det1," Plant Cell 7:117-129 (1995), which is hereby incorporated by reference in its entirety) and the barley BEL, JUBEL2 (Müller et al., "In vitro Interactions Between Barley TALE Homeodomain Proteins Suggest a Role for Protein-protein Associations in the Regulation of Knox Gene Function," Plant J 27:13-23 (2001), which is hereby incorporated by reference in its entirety). Protein interaction using the two-hybrid system demonstrated that the first 80 aa of this domain (up to QVKAT of the STBEL-05 sequence and comprising the first two predicted helices of this region) are necessary to mediate interaction with POTH1 (interaction of construct pAD5-9 with POTH1). Deletion of as little as the first 20 aa of this domain (comprising a major portion of the first predicted helix) interfered with the interaction with POTH1 (Figures 13B and 10B, construct pAD5-2). The results also showed that deletion of 43 aa from the carboxy-end of the 120-aa domain (see Figure 10B, construct pAD5-9; comprising the third helical structure) did not affect protein interaction. Deletion of the two carboxyl-terminal helices in this region (construct pAD5-11) resulted in a loss of interaction. It appears that all three helical structures contribute to specific binding affinity for POTH1 but that only the amino-terminal two-thirds of the 120-aa domain are necessary for binding to occur. Müller et al., "In vitro Interactions Between Barley TALE Homeodomain Proteins Suggest a Role for Protein-protein Associations in the Regulation of Knox Gene Function," Plant J 27:13-23 (2001), which is hereby incorporated by reference in its entirety, identified a coiled-coil region in a BEL protein of barley

that was involved in the interaction with KNOX proteins. This coiled-coil domain overlaps with 48 of the 80 aa (and comprising the first helix) identified as essential for interaction to occur.

[0174] Sequence differences in this putative protein-binding region appear  
5 to contribute to the regulation of POTH1 activity by affecting binding affinity to a shared partner. In the interaction between PIF3, a basic helix-loop-helix factor, and phytochrome A and B, phytochrome B has tenfold greater binding affinity for the PIF3 partner than phytochrome A (Zhu et al., “Phytochrome B Binds With Greater Affinity Than Phytochrome A to the Basic Helix-loop-helix Factor PIF3  
10 in a Reaction Requiring the PAS Domain of PIF3,” Proc Natl Acad Sci USA 97:13419-13424 (2000), which is hereby incorporated by reference in its entirety). A comparison of this 120-aa domain in the potato BELs revealed that StBEL-05 (expressed ubiquitously) has a 58 % similarity match to StBEL-13 (expressed predominately in the SAM and flower only) and that StBEL-13 has a 63 % match  
15 to StBEL-30 in this conserved region. Such differences in sequence may mediate binding affinities to shared partners and, coupled with expression patterns, could reflect organ-specific differences in function.

[0175] Conservation in sequence among these seven proteins was also identified in two short amino acid sequence motifs, one near the carboxyl-end of  
20 the protein (VSLTLGL) (SEQ ID NO:15) and another just upstream of the BELL domain (SKY box, Figure 13A). Both of these regions are conserved among other plant BELs. Protein interaction studies showed that the VSLTLGL (SEQ ID NO:15) box is not involved in protein interaction with POTH1 and its function remains unknown. Consistent with Bellaoui et al., “The Arabidopsis BELL1 and  
25 KNOX TALE Homeodomain Proteins Interact Through a Domain Conserved Between Plants and Animals,” Plant Cell 13:2455-2470 (2001), which is hereby incorporated by reference in its entirety, it was observed that, whereas binding occurred without the 229 aa at the amino terminus of StBEL-05 (construct pAD5-1), this 229 aa sequence alone, containing the SKY box, was sufficient to mediate  
30 an interaction with POTH1 (and other class I KNOX proteins). This 229-aa sequence, however, did not interact with a class II KNOX protein. Müller et al., “In vitro Interactions Between Barley TALE Homeodomain Proteins Suggest a

Role for Protein-protein Associations in the Regulation of Knox Gene Function,”  
Plant J 27:13-23 (2001), which is hereby incorporated by reference in its entirety,  
identified the SKY-box sequence in the barley BEL protein to be a part of the  
KNOX-interacting domain. Our deletion analysis indicates that the SKY box  
5 enhances the binding affinity of StBEL-05 to KNOX partners.

**Example 31 – Discussion: The Protein Binding Region of POTH1**

[0176] In addition to the homeodomain, KNOX TFs also contain a  
conserved region of approximately 100 aa, upstream from the homeodomain,  
10 known as the KNOX (MEINOX) domain, and postulated to be involved in  
protein/protein interaction (Bürglin, “The PBC Domain Contains a MEINOX  
Domain: Coevolution of Hox and TALE Homeobox Genes,” Dev Genes Evol  
208:113-116 (1998), which is hereby incorporated by reference in its entirety).  
Using deletion mutants in the two-hybrid yeast system, regions of amino acid  
15 sequence in the KNOX domain of the class I KNOX protein, POTH1, that are  
involved in an interaction with the BEL TFs have been identified. Binding to the  
BEL partner is mediated by the KNOX domain but is not dependent on the  
presence of the first half of the 120 aa KNOX region (Figure 10A). Similar  
results were obtained by Müller et al., “In vitro Interactions Between Barley  
20 TALE Homeodomain Proteins Suggest a Role for Protein-protein Associations in  
the Regulation of Knox Gene Function,” Plant J 27:13-23 (2001), which is hereby  
incorporated by reference in its entirety. Sakamoto et al., “The Conserved KNOX  
Domain Mediates Specificity of Tobacco KNOTTED1-type Homeodomain  
Proteins,” Plant Cell 11:1419-1431 (1999), which is hereby incorporated by  
25 reference in its entirety, showed by using chimeric proteins that the second half of  
the KNOX domain (designated KNOX2) of a tobacco KNOX protein (NTH15,  
with 63 % similarity to POTH1 in the KNOX region) was most important for  
determining the severity of the mutant phenotype. Their results indicated that this  
conserved domain was even more important in determining the phenotype than  
30 the DNA-binding domain. The deletion analysis for POTH1 in the present study  
combined with the results of Sakamoto et al., “The Conserved KNOX Domain  
Mediates Specificity of Tobacco KNOTTED1-type Homeodomain Proteins,”

Plant Cell 11:1419-1431 (1999), which is hereby incorporated by reference in its entirety, indicate that the interaction of the BEL proteins with the KNOX domain is a prominent control mechanism for mediating KNOX activity and maintaining stable development of the vegetative meristem. KNOX2 contains 18 aa that are

5 predicted to form an  $\alpha$ -helix and are conserved among all tobacco and potato KNOX proteins. POTH1 has a close sequence match to members of the family of KNOX proteins of tobacco (Nishimura et al., Over-expression of Tobacco Knotted1-type Class1 Homeobox Genes Alters Various Leaf Morphology," Plant Cell Physiol 41:583-590 (2000), which is hereby incorporated by reference in its

10 entirety), with an overall sequence similarity ranging from 60 to 73 % and an even greater match in the conserved KNOX and homeodomain regions. Using the two-hybrid system, all seven BELs of potato interacted with four other tobacco class I-type KNOX proteins. Unlike KNOX proteins of barley (Müller et al., "In vitro Interactions Between Barley TALE Homeodomain Proteins Suggest a Role for

15 Protein-protein Associations in the Regulation of Knox Gene Function," Plant J 27:13-23 (2001), which is hereby incorporated by reference in its entirety) and rice (Nagasaki et al., "Functional Analysis of the Conserved Domains of a Rice KNOX Homeodomain Protein, OSH15," Plant Cell 13:2085-2098 (2001), which is hereby incorporated by reference in its entirety), however, POTH1 did not form

20 homodimers *in vitro*. Structural similarities to the MEIS domain of animal homeodomain proteins (Bürglin, "The PBC Domain Contains a MEINOX Domain: Coevolution of Hox and TALE Homeobox Genes," Dev Genes Evol 208:113-116 (1998), which is hereby incorporated by reference in its entirety) suggest that sequences in the KNOX domain of plants are important for

25 interactions with other proteins (Sakamoto et al., "The Conserved KNOX Domain Mediates Specificity of Tobacco KNOTTED1-type Homeodomain Proteins," Plant Cell 11:1419-1431 (1999), which is hereby incorporated by reference in its entirety). These results confirm the function of this domain in an interaction with a BEL1-like protein of potato.

30

**Example 32 – Discussion: The Function of the BEL/POTH1 Interaction**

[0177] Through both molecular and genetic analyses, the BEL proteins are known to function in the development of ovules. Reiser et al., “The BELL1 Gene Encodes a Homeodomain Protein Involved in Pattern Formation in the

5 *Arabidopsis* Ovule Primordium,” Cell 83:735-742 (1995), which is hereby incorporated by reference in its entirety, showed that *BELL1* of *Arabidopsis* was involved in the pattern formation of ovule primordium. More specifically, the expression of NOZZLE (a nuclear protein and putative TF) and BELL are spatially linked and interact with other transcription factors to determine distal-

10 proximal pattern formation during ovule development (Balasubramanian et al., “NOZZLE Links Proximal-Distal and Adaxial-Abaxial Pattern Formation During Oovule Development in *Arabidopsis thaliana*,” Development 129:4291-4300 (2002), which is hereby incorporated by reference in its entirety). Both *NOZZLE* and *BELL* are chalazal identity genes that share overlapping functions

15 (Balasubramanian et al., “NOZZLE Regulates Proximal-Distal Formation, Cell Pproliferation and Early Sporogenesis During Oovule Development in *Arabidopsis thaliana*,” Development 127:4227-4238 (2000), which is hereby incorporated by reference in its entirety). In *bell* mutants, the chalazal domain undergoes altered development and growth of the integuments is replaced by

20 irregular outgrowths (Mondrusan et al., “Homeotic Transformation of Ovules into Carpel-like Structures in *Arabidopsis*,” Plant Cell 6:333-349 (1994), which is hereby incorporated by reference in its entirety). Overexpression of an apple BEL gene (*MDH1*) in *Arabidopsis* produced plants that were dwarf, had reduced fertility, and exhibited changes in both carpel and fruit shape (Dong et al.,

25 “MDH1: an Apple Homeobox Gene Belonging to the BEL1 Family,” Plant Mol Biol 42:623-633 (2000), which is hereby incorporated by reference in its entirety). Overall, these results support that BEL proteins function in controlling the formation of carpellate tissues and plant fertility. Overexpression of a cDNA of a barley BEL in tobacco produced plants that were dwarf and exhibited malformed

30 leaves and reduced apical dominance (Müller et al., “In vitro Interactions Between Barley TALE Homeodomain Proteins Suggest a Role for Protein-protein Associations in the Regulation of Knox Gene Function,” Plant J 27:13-23 (2001),

- which is hereby incorporated by reference in its entirety). This BEL1-like cDNA isolated from floral meristems produced a sense phenotype similar to a class I *knox* overexpresser (Chan et al., “Homeoboxes in Plant Development,” Biochim Biophys Acta 1442:1-19 (1998), which is hereby incorporated by reference in its entirety). All seven of the BEL TFs in this study were isolated from stolons, a vegetative organ. Based on these results and the patterns of mRNA accumulation in potato, it appears that the BEL1 TFs of potato play a diverse role in plant growth by regulating the development of both reproductive and vegetative meristems.
- 10 [0178] Because the BEL TFs of potato and POTH1 interact, the function of one provides a clue to the function of the other. The KNOX protein of tobacco, NTH15, affects plant growth by regulating GA levels through a direct interaction with a specific motif in regulatory sequences of the GA 20-oxidase1 gene, a key GA biosynthetic enzyme (Sakamoto et al., KNOX Homeodomain Protein Directly
- 15 Suppresses the Expression of a Gibberellin Biosynthesis Gene in the Tobacco Shoot Apical Meristem,” Genes Dev 15:581-590 (2001), which is hereby incorporated by reference in its entirety). NTH15 directly suppresses the expression of GA 20-oxidase1 within specific cells of the SAM to maintain the indeterminate state of corpus cells. The *knotted1*-like protein of potato, POTH1,
- 20 is also involved in the regulation of GA synthesis and acts as a developmental switch during tuber formation. Transgenic plants that overexpressed *POTH1* had reduced levels of GA 20-oxidase1 mRNA, altered levels of GA intermediates, and exhibited a phenotype that could be partially rescued by GA<sub>3</sub> treatment (see above). These plants were dwarf and developed malformed leaves. Under both
- 25 short-day (inductive conditions) and long-day (noninductive) photoperiods, *POTH1* overexpressing lines produced more tubers than controls (see above). These sense lines exhibited a capacity for enhanced tuber formation. Lines that overexpressed *StBEL-05* produced tubers even under LD *in vitro* conditions, whereas control plants produced tubers only after 10 days of SD conditions.
- 30 Overall, the BEL sense lines produced more tubers at a faster rate than controls even on soil-grown plants. After 14 days of SD conditions, soil-grown *StBEL-05* overexpressers exhibited a threefold increase in tuber production relative to wild-

type plants (Table 2). Thus, both POTH1 and StBel-05 overexpression lines produced more tubers at a faster rate than controls (see Figures 17A-D). In Figure 17D, stolon tips excised from *in vitro* plantlets overexpressing *POTH1* that were not tuberizing were cultured. After a 20-day incubation in the dark on 8% (w/v) sucrose, stolons from all five *POTH1* sense lines produced more tubers than wild-type stolons. Line 11 exhibited almost a 10-fold increase in tuber yield (262 mg stolon tip<sup>-1</sup>) after 35 days in culture compared with wild-type plants (27 mg stolon tip<sup>-1</sup>).

[0179] All of the above results show that that the expression of both POTH1 and its protein partner, STBEL-05, is associated with an enhanced rate of tuber formation. In addition to enhanced tuber production, select *StBEL-05* lines exhibited increases in cytokinin levels and a reduction in GA 20-oxidase1 mRNA similar to *POTH1* overexpression lines. This increase in cytokinin levels could explain the enhanced rate of growth for the *StBEL-05* lines, although excessive accumulation may have led to the reduction in growth exhibited by mature plants of lines 11 and 20. GA is involved in regulating cell growth in a tuberizing stolon (Xu et al., "The Role of Gibberellin, Absciscic Acid, and Sucrose in the Regulation of Potato Tuber Formation *in vitro*," Plant Physiol 117:575-584 (1998), which is hereby incorporated by reference in its entirety) and in contributing to the control of the photoperiodic response of tuber formation (Jackson et al., "Control of Tuberisation in Potato by Gibberellins and Phytochrome," B. Physiol Plant 98:407-412 (1996), Martínez-García et al., "The Interaction of Gibberellins and Photoperiod in the Control of Potato Tuberization," J Plant Growth Regul 20:377-386 (2001), which are hereby incorporated by reference in their entirety). Low levels of GA in the stolon tip are correlated with tuber induction (Xu et al., "The Role of Gibberellin, Absciscic Acid, and Sucrose in the Regulation of Potato Tuber Formation *in vitro*," Plant Physiol 117:575-584 (1998), which is hereby incorporated by reference in its entirety). Tuberization is also affected by cytokinin accumulation, with high levels inhibiting and moderate levels promoting tuber formation (Gális et al., "The Effect of an Elevated Cytokinin Level Using the *ipt* Gene and N<sup>6</sup>-Benzyladenine on Single Node and Intact Potato Plant Tuberization *in vitro*," J Plant Growth Regul 14:143-150 (1995); Romanov et al.,

“Effect of Indole-3-Acetic Acid and Kinetin on Tuberisation Parameters of Different Cultivars and Transgenic Lines of Potato *in vitro*,” Plant Growth Reg 32:245-251 (2000), which are hereby incorporated by reference in their entirety). Local accumulation of cytokinins in axillary buds of transgenic tobacco produced truncated, tuberizing lateral branches (Guivarc’h et al., “Local Expression of the *ipt* Gene in Transgenic Tobacco (*Nicotiana tabacum* L. cv. SR1) Axillary Buds Establishes a Role for Cytokinins in Tuberization and Sink Formation,” J Exp Bot 53:621-629 (2002), which is hereby incorporated by reference in its entirety). Through an interaction with POTH1, the BEL protein encoded by *StBEL-05* may also function to regulate hormone levels in stolons or leaves to favor the formation of tubers.

[0180] The results set forth above indicate that the physical interaction between the KNOX and BEL proteins provides a molecular basis for regulating processes of growth in the potato and that overexpression of each partner alone affects vegetative development and enhances tuber formation.

**Example 33 -- Both POTH1 and StBEL-05 Interact to Repress Transcriptional Activity of the GA20 Oxidase1 Gene of Potato - Preliminary Results**

[0181] If POTH1 and StBEL physically interact and their overexpression produces transgenic plants that exhibit similar developmental pathways, it is reasonable to assume that they target the same gene. Using gel mobility shift assays (Figure 18), it is shown that in tandem POTH1 and StBEL-05 bind to the P1 region of the GA20 oxidase1 promoter. In tandem, StBEL-05 and POTH1 had a greater binding affinity for the *ga20ox1* promoter than either alone. The StBEL-05-POTH1 heterodimer bound specifically to a composite sequence TTGACTTGAC (SEQ ID NO: 20) containing two adjacent TGAC cores in the P1 region. Using a transcription assay with GUS reporter driven by the *ga20ox1* promoter in tobacco protoplasts, StBEL-05 and POTH1 alone suppressed the activity of the *ga20ox1* promoter by more than 50%, together about 80%. The binding affinity of POTH1 and StBEL-05 represses the transcriptional activity of the promoter (Figure 19).



[0182] Consistent with the *in vitro* results of StBEL/POTH1 repression of the GA20 oxidase1 promoter/GUS marker (Figure 19), GA20 oxidase1 mRNA levels are also reduced in stolons of the StBEL-05 sense lines grown under long days (Figure 20). This reduction in mRNA will lead to a reduction in bioactive  
5 GA and result in facilitating tuber formation. StBEL-05 mRNA levels were found to increase in both stolons and leaves of WT plants in response to the inductive conditions of short days. These results are consistent with the proposed role of GA in mediating photoperiodic responses in potato (Martinez-Garcia et al., "The Interaction of Gibberellins and Photoperiod in the Control of Potato  
10 Tuberization," J. Plant Growth Regul. 20:377-386 (2002), which is hereby incorporated by reference in its entirety).

[0183] These preliminary data show that POTH1 and StBEL-05 proteins interact *in vitro* and that overexpression of each separately, produces plants that are enhanced in their capacity to form tubers. Both proteins interact to repress the  
15 transcriptional activity of a key GA biosynthetic gene. Because expression of the BEL TFs appears to be differential, the BELs appear to act in tandem with POTH1 (or other KNOX proteins) to regulate growth differently in the various organs or cells of the potato. A more detailed description of the above experiments is provided in Examples 34-43, below.

20

**Example 34 -- BEL and KNOX Interaction Mediates Transcriptional Activity of the Potato *ga20ox1* Promoter - Plant Materials**

[0184] Tobacco 'Petit Havana' plants were maintained in Murashige and Skoog basal medium (1962) supplemented with 2% sucrose and incubated at 25  
25 °C, under 16 hour photoperiods for three to four weeks.

**Example 35 -- BEL and KNOX Interaction Mediates Transcriptional Activity of the Potato *ga20ox1* Promoter - Protein Expression and Purification in *E. coli***

30 [0185] Glutathione S-transferase (GST) fusion constructs were generated by introducing full-length cDNAs of *StBEL-05* and *POTH1* in frame with GST into the pGEX-5X-2 expression vector (Roche, Indianapolis, IN) and transformed

into BL21 (DE3) *E. coli* cells (Stratagene, La Jolla, CA). Cells were grown at 30 °C until the OD<sub>600</sub> reached 0.6, induced with 1.0-mM isopropyl-β-D-thiogalactopyranoside, and cultured for 5 hours. The manufacturer's protocol (Roche) was followed for cell lysis and affinity purification by using glutathione  
5   sepharose 4B beads. The GST portion of the fusion protein was cleaved by Factor Xa protease (Promega, Madison, WI). Purified StBEL-05 and POTH1 protein were frozen in liquid N<sub>2</sub> and stored at -80 °C.

10   **Example 36 -- BEL and KNOX Interaction Mediates Transcriptional Activity of the Potato *ga20ox1* Promoter - Gel Retardation Assay**

[0186]       The first intron with partial flanking exon sequence (450 bp) of potato *ga20ox1* and its promoter (981bp, provided by Dr. Salomé Prat, CSIC Cantoblanco Campus, Univ. of Madrid, Spain) were used for gel mobility shift assays. Polymerase chain reaction (PCR) was used to amplify three regions of the  
15   promoter: -981 to 636 (P1), -660 to 307 (P2), and -331 to 0 (P3). About a 25-bp overlap was maintained between P1 and P2 or P2 and P3 in the chance that the protein-binding site would span the overlapped region. The first intron of this gene was amplified from potato genomic DNA by using PCR and the oligos 5'-GGATCCTTGAAGTGGCTCTTCTCT-3' (SEQ ID NO:21) and 5'-  
20   AATCTAGAGACACTCTCTTTTTCGT-3' (SEQ ID NO:22) as primers. These primers were designed based on the site of the first intron of the tobacco GA20 oxidase gene *Ntc12*. The four fragments were purified on a 1.4% agarose gel and labeled with α<sup>32</sup>P-dATP using Klenow fragment. DNA-binding reactions were set up on ice in 20 μL containing 10-mM Tris-HCl (pH 7.5), 5% glycerol, 0.5-mM  
25   EDTA, 0.5-mM DTT, 0.05% NP-40, 50-mM NaCl, 50-mg • L<sup>-1</sup> poly (dG-dC) • poly (dG-dC) (Amersham Pharmacia Biotech, Piscataway, NJ), 100-ng protein, and 1-fmol labeled DNA. After incubation on ice for 30 minutes, the reactions were resolved on a 6 % native polyacrylamide gel in 1X TGE (Tris-Glycine-EDTA) buffer. The gel was dried and exposed to X-ray film.

30   [0187]       In the competition assays, unlabeled double-stranded DNA fragments (10X, 25X, 50X, 100X) were incubated with the recombinant protein before the addition of the radioactive probe. The dissociation rates were

determined by adding 500-fold more cold DNA fragments to the DNA-binding reactions that were being incubated on ice, and loaded onto the running gel every 10 minutes. Mutated oligos for binding sites were synthesized by the DNA Sequencing and Synthesis Facility, Iowa State University (Ames, IA).

5

**Example 37 – BEL and KNOX Interaction Mediates Transcriptional Activity of the Potato *ga20ox1* Promoter - Transcription Assay**

*Generation of Reporters and Effectors*

[0188] The cauliflower mosaic virus (CaMV) 35S promoter in pBI221  
10 (Clontech, Palo Alto, CA) was replaced by an enhancer fragment (-832 to -50) of the 35S promoter plus 980 bp of the *ga20ox1* promoter to generate the pGAOP:: $\beta$ -glucuronidase (GUS) reporter construct. With this construct, the reporter GUS transcription level is augmented but its transcription may still be affected by the *ga20ox1* promoter. A CaMV 35S promoter-driven luciferase (LUC) construct  
15 35S-LUC (obtained from Dr. Takahashi, Dept. of Biological Sciences, Graduate School of Science, Univ. of Tokyo, Japan) was used as an internal control. Effector constructs were also generated by using pBI221 vector as a backbone, with the GUS gene replaced by the full-length cDNAs of either *StBEL-05* or *POTH1*, downstream of the CaMV 35S promoter. Truncated cDNAs that encode  
20 the N-terminal protein-binding domains of *StBEL-05* or *POTH1* were used to generate the dominant negative constructs, *StBEL5 $\Delta$ C295* and *POTH1 $\Delta$ C122*, respectively. The reporter construct with the mutated promoter was generated by site-directed PCR mutagenesis with oligos 5'-  
CTATTTGACTTC\*ACACGGTTATTT-3' (SEQ ID NO:23) and 5'-  
25 AAATAACCGTGTG\*AAGTCAAATAG-3' (SEQ ID NO:24).

*Transfection Assay*

[0189] Fully expanded leaves from three- to four-week-old tobacco plants were excised and placed in K3 basal media (Kao et al., "Nutritional Requirements  
30 for Growth of *Vicia hajastana* Cells and Protoplasts at a Very Low Density in Liquid Media," Planta 126:105-110 (1975), which is hereby incorporated by

reference in its entirety) supplemented with 0.4 M sucrose, 0.25% (w-v) cellulases (Karlan Research Products, Santa Rosa, CA), and 0.05% (w-v) macerases (Calbiochem, La Jolla, CA) and incubated for overnight at 28 °C. After incubation, the liberated protoplasts were filtered through sterile cheesecloth into a Babcock bottle, and centrifuged for 10 minutes at 1000 rpm. Protoplasts were collected from the bottleneck area and washed once in K3 media with 0.4 M sucrose and resuspended in K3 media containing 0.4 M glucose to a final concentration of  $4 \times 10^6$  protoplasts per milliliter.

[0190] For each transfection analysis, 700  $\mu$ L of tobacco protoplasts (prepared as described above) were mixed with 30  $\mu$ L 2 M KCl and plasmid DNA in an electroporation cuvette with 0.4-cm electrode gap. The plasmid DNA was a mixture of 2  $\mu$ g of the pGAOP::GUS reporter construct, 0.1  $\mu$ g of the 35S-LUC construct as internal control, and a different combination of 2  $\mu$ g of each effector plasmid. After electroporation (voltage = 170 V, capacitance = 125  $\mu$ F, Gene Pulser Transfection Apparatus; Bio-Rad, Hercules, CA), 4.0 mL of Murashige and Skoog (1962) basal media was added, and the protoplasts were incubated in the dark at room temperature for 40 to 48 hours before conducting GUS and LUC activity assays. Transfections were performed three times for each effector combination.

[0191] Luciferase assays were performed by injecting 100- $\mu$ L luciferase substrate (Promega, Madison, WI) into 20  $\mu$ L of extract and measuring the emitted photons for 15 seconds in a TD-20 luminometer (Turner Designs, Sunnyvale, CA). Fluorometric GUS assays were performed as described (Jefferson, "Assaying Chimeric Genes in Plants: The GUS Gene Fusion System," Plant Mol. Biol. Rep. 5:387-405 (1987), which is hereby incorporated by reference in its entirety). A fluorescence multiwell plate reader, Fluoroskan II (MTX labs, Vienna, VA), was used to measure GUS activity at 365 nm (excitation) and 455 nm (emission). Each sample was measured three times for both LUC and GUS activity. Relative GUS-LUC activity was calculated by dividing the ratio of GUS activity to LUC activity from different effectors with the ratio from reporter plasmid alone. Relative activities calculated from three transfection replications were presented as a mean  $\pm$  SE.

**Example 38 – Results: StBEL-05 and POTH1 Bind to the Regulatory Regions of *ga20ox1***

- 5      **[0192]**      Recombinant StBEL-05 protein expressed from *E. coli* retarded the mobility of all three promoter sequences and the first intron (Figures 18A and B). POTH1 only formed a complex with P1. StBEL-05 and POTH1 together produced a supershifted band with P1, which had stronger signal intensity and migrated much slower than either the StBEL-05-P1 or POTH1-P1 complexes (Figure 18A). Competition assays were performed with labeled P1 and unlabeled
- 10      P1 or unlabeled P3. With increased unlabeled P1, the P1-StBEL-05 complex quickly disappeared (Figure 21A). With unlabeled P3, however, even at a concentration 100-fold more than labeled P1, the shifted band was still present (Figure 21A). Unlabeled P1 also reduced the P1-POTH1 complex formation, but unlabeled P3 had no effect on the P1-POTH1 complex (Figure 21B).
- 15      **[0193]**      Consistent with the increased signal intensity of the StBEL-05-POTH1-P1 complex, the dissociation rate of this complex was much slower than either the StBEL-05-P1 or POTH1-P1 complexes (Figure 22). Although StBEL-05 could bind to P2, P3, and the intron fragments, there was no supershifted band formed when both StBEL-05 and POTH1 were incubated with these three DNA
- 20      fragments (Figure 18A). These results indicate that both StBEL-05 and POTH1 are required for binding to the P1 DNA fragment. Based on these results, at least two TALE homeodomain binding sites may be present in P1. To support this premise, excessive amounts of a truncated protein containing only the HD portion of StBEL-05 produced a supershifted band similar to the POTH1-StBEL-05-P1
- 25      complex. Apparently, there were two binding sites recognized by StBEL-05 in P1. No supershifted band was detected, however, when P1 was incubated with excessive amounts of full-length StBEL-05 or POTH1. This indicates that the two binding sites in P1 are in close proximity to one other and that two full-length StBEL-05 molecules cannot bind to both sites at the same time because of size
- 30      constraints.

**Example 39 – Results: The StBEL-05-POTH1 Heterodimer Binds Specifically to the TGA(C/G)(T/A)TGAC Site**

[0194] Based on the *Arabidopsis* KNOX-BEL heterodimer binding site TGACAG(G/C)T (SEQ ID NO:25) (Smith et al., “Selective Interaction of Plant  
5 Homeodomain Proteins Mediates High DNA-Binding Affinity,” Proc. Natl. Acad. Sci. 99:9579-9584 (2002), which is hereby incorporated by reference in its entirety) and the TGAC binding core confirmed for MEINOX proteins (Smith et al., “Selective Interaction of Plant Homeodomain Proteins Mediates High DNA-Binding Affinity,” Proc. Natl. Acad. Sci. 99:9579-9584 (2002); Tejada et al.,  
10 “Determinants of the DNA-Binding Specificity of the Avian Homeodomain Protein, AKR,” DNA and Cell Biol. 18:791-804 (1999), which are hereby incorporated by reference in their entirety, one putative site, TTGACTTGAC (SEQ ID NO:20), in the potato *ga20ox1* promoter P1 region was identified. Oligonucleotides with serial point mutations across this site were used as probes  
15 in gel-retardation assays in the presence of StBEL-05, POTH1, or both. Point mutations across this site did not affect the binding of either StBEL-05 or POTH1 alone, but most mutations in TGACTTGAC (SEQ ID NO:26) abolished the binding by StBEL-05-POTH1 heterodimer. Based on these results, it was deduced that the consensus sequence of the StBEL-05-POTH1 heterodimer is  
20 TGA(C/G)(T/A)TGAC (SEQ ID NO:27).

**Example 40 – Results: Repression of *ga20ox1* Promoter Requires the Interaction of StBEL-05 and POTH1**

[0195] *POTH1* encodes for a 345-residue protein estimated to have a mass  
25 of 37.95 kDa. The coding sequence of the protein includes the 97-aa KNOX domain and the 64-aa homeodomain consisting of three helices (Figure 23A). The KNOX domain of POTH1 contains two conserved regions, designated Knox I and II. StBEL-05 is 688 aa in length with an estimated mass of 75.68 kDa. The coding sequence of StBEL-05 contains the conserved sky box, BELL domain,  
30 homeodomain, and the proline-tyrosine-proline (P-Y-P) loop between helices I and II (Figure 23B). The BELL domain is 120 aa in length and the HD of StBEL-05 is 61 aa.

[0196] When co-transfected with effector p35S::StBEL5, p35S::POTH1, or both (Figure 24A), relative GUS-LUC activity of the pGAOP::GUS reporter construct decreased by more than half (Figure 24B). Neither StBEL-05 nor POTH1 showed any effect on the activity of the CaMV 35S promoter (Figure 24C). To eliminate the possibility that endogenous BEL1-like or KNOX proteins cooperatively interact with POTH1 or StBEL-05, respectively, truncated forms of StBEL-05 and POTH1, StBEL5 $\Delta$ C295 and POTH1 $\Delta$ C122 (Figure 25A), were generated to use as dominant negatives in the transcription assays. StBEL5 $\Delta$ C295 and POTH1 $\Delta$ C122 contain the intact protein-binding domain, but lack the carboxy-terminal region including the homeodomain. StBEL5 $\Delta$ C295 and POTH1 $\Delta$ C122 can interact with endogenous KNOX or BEL1-like proteins, respectively. Such heterodimers are not functional due to the lack of the homeodomain from the truncated proteins. In transcription assays with pGAOP::GUS as reporter, StBEL5 $\Delta$ C295 had little effect on the activity of the *ga20ox1* promoter (Figure 25B). When co-transfected with StBEL-05, StBEL5 $\Delta$ C295 abolished almost all of the repression activity of StBEL-05 (Figure 25B). POTH1 $\Delta$ C122 had a similar effect on the repression activity of POTH1 (Figure 25C).

20 **Example 41 -- Results: The Binding Site in the *ga20ox1* Promoter Acts as a *cis*-element for the Repression by StBEL-05-POTH1 Heterodimer**

[0197] To investigate whether the StBEL-05-POTH1 binding site identified through EMSA studies functions as a *cis*-element, a reporter construct with a point mutation in the binding site was used for the transcription assay (Figure 26A). Constructs containing this single mutation exhibited no detectable repression of promoter activity when co-transfected with either StBEL-05, POTH1, or both (Figures 26B-C).

30 **Example 42 -- Discussion: Cooperative Interaction Between StBEL-05 and POTH1 Mediates Binding Affinity for the *ga20ox1* Promoter**

[0198] To regulate target gene expression, a transcription factor binds to the regulatory sequence of its target gene or interacts with another protein that

does. Gel-retardation assays showed that both StBEL-05 and POTH1 bound to the promoter region of potato *ga20ox1* gene, and StBEL-05 could also bind with the first intron sequence (Figures 18A-B). Unlabeled P3 competed with the StBEL-05-P1 complex, but not as effectively as unlabeled P1 (Figure 21A),  
5 whereas P3 had no competition effect with the POTH1-P1 complex (Figure 21B). These results indicated that the interaction between these two TALE HD proteins and P1 was specific and that StBEL-05 bound to P1 more strongly than to P3. It is highly likely then that P1 contains the *cis* element that functions with this protein complex in planta. The tobacco KNOX protein, NTH15, binds to both the  
10 promoter and the first intron of *GA20 oxidase*, but with higher affinity to the first intron (Sakamoto et al., “KNOX Homeodomain Protein Directly Suppresses the Expression of a Gibberellin Biosynthetic Gene in the Tobacco Shoot Apical Meristem,” Genes & Dev. 15:581-590 (2001), which is hereby incorporated by reference in its entirety). NTH15 is not the tobacco homolog of POTH1 and this  
15 may explain the disparity in binding affinities. No BEL partners were tested for binding with the tobacco KNOX protein or the *GA20 oxidase* promoter.

[0199] Several consensus binding sites for KNOX proteins have been identified from either target gene promoters or *in vitro* binding site selection by using KNOX HD proteins from barley (Krusell et al., “DNA Binding Sites  
20 Recognized in Vitro by a Knotted Class 1 Homeodomain Protein Encoded by the Hooded Gene, *K*, in Barley (*Hordeum vulgare*),” FEBS Lett. 408:25–29 (1997), which is hereby incorporated by reference in its entirety, tobacco (Sakamoto et al., “KNOX Homeodomain Protein Directly Suppresses the Expression of a Gibberellin Biosynthetic Gene in the Tobacco Shoot Apical Meristem,” Genes &  
25 Dev. 15:581-590 (2001), which is hereby incorporated by reference in its entirety), and rice (Nagasaki et al., “Functional Analysis of the Conserved Domains of a Rice KNOX Homeodomain Protein, OSH15,” Plant Cell 13:2085-2098 (2001), which is hereby incorporated by reference in its entirety). Because the homeodomains, especially the third  $\alpha$ -helix in the HD region, of these KNOX  
30 proteins are almost identical, the consensus sequences recognized by them share a core TGTCAC motif (Nagasaki et al., “Functional Analysis of the Conserved Domains of a Rice KNOX Homeodomain Protein, OSH15,” Plant Cell 13:2085-



2098 (2001), which is hereby incorporated by reference in its entirety). Two interacting TALE proteins of vertebrates, Meis1 and Pbx1, dimerize on the composite DNA sequence, TGATTGACAG (SEQ ID NO:28), containing 5'-Pbx and 3'-Meis half sites (Chang et al., "Meis Proteins are Major in Vivo DNA Binding Partners for Wild-Type But Not Chimeric Pbx Proteins," Mol. Cell. Biol. 7:5679-5687 (1997), which is hereby incorporated by reference in its entirety). Using random oligonucleotide selection, the consensus sequence, TGACAG(G/C)T (SEQ ID NO:25), was identified for the *Arabidopsis* BEL-KNOX heterodimeric complex (Smith et al., "Selective Interaction of Plant Homeodomain Proteins Mediates High DNA-Binding Affinity," Proc. Natl. Acad. Sci. 99:9579-9584 (2002), which is hereby incorporated by reference in its entirety). Because the StBEL-05-POTH1-P1 complex requires both proteins to bind the target DNA, and increased amounts of the StBEL-05 homeodomain lead to a supershifted band, this indicates that there are two closely located TALE homeodomain binding sites in the P1 region similar to the two half binding sites for Meis1 and Pbx1 (Chang et al., "Meis Proteins are Major in Vivo DNA Binding Partners for Wild-Type But Not Chimeric Pbx Proteins," Mol. Cell. Biol. 7:5679-5687 (1997), which is hereby incorporated by reference in its entirety). Based on these results and comparisons to the known binding motifs, a potential StBEL5-POTH1 binding site, TTGACTTGAC (SEQ ID NO:25), has been identified in the P1 fragment. Gel-retardation assays confirmed that this oligo was sufficient for binding to StBEL-05, POTH1, and StBEL5-POTH1. Mutational gel-retardation analysis of this BEL-KNOX binding site showed that the StBEL-05-POTH1 heterodimer recognizes the 9-bp sequence, TGA(C/G)(T/A)TGAC (SEQ ID NO:27), containing two TGAC cores. StBEL-05 and POTH1 could bind to either one of the TGAC cores, because serial mutations had no effect on the DNA-binding ability of StBEL-05 or POTH1.

[0200] It has been a paradox for HD proteins regarding their high level of functional specificity in directing developmental programs and their high degree of redundancy in binding site specificity. Besides the low affinity and high redundancy in binding sites, the 5-base consensus sequences recognized by HD proteins randomly show up on average once every 1.0 kb in eukaryotic genomes

(Mann et al., "Extra Specificity From Extradenticle: The Partnership Between Hox and Exd-Pbx Homeodomain Proteins. Trends Genet. 12:258-262 (1996), which is hereby incorporated by reference in its entirety). Therefore, it is likely that interaction with other DNA-binding transcription factors is necessary for HDs  
5 to affect binding affinity and specificity. Monomeric HD proteins have modest specificity for DNA binding, but their specificity is greatly increased through cooperative binding with other DNA binding partners (Mann et al., "Extra Specificity From Extradenticle: The Partnership Between Hox and Exd-Pbx Homeodomain Proteins. Trends Genet. 12:258-262 (1996), which is hereby  
10 incorporated by reference in its entirety). The gel-retardation assays also showed that StBEL-05 and POTH1 in tandem formed a complex with P1 with greater signal intensity than either POTH1-P1 or StBEL5-P1 complexes (Figure 18A), and that the StBEL-05-POTH1-DNA complex had a much slower dissociation rate (Figure 22). Both of these results indicate that the BEL-KNOX heterodimer  
15 has an increased binding affinity for the target site.

**Example 43 -- Discussion: STBEL-05-POTH1 Heterodimer Mediates the Repression of the *ga20ox1* Promoter**

[0201] The previous examples showed that both *StBEL-05* and *POTH1*  
20 overexpression mutants exhibited decreased *ga20ox1* mRNA levels in stolons and leaves, respectively (see Examples 1-32). Gel-retardation assay results showed that these two transcription factors bound to the promoter and the first intron of *ga20ox1*. These results indicate that StBEL-05 and POTH1 directly represses  
25 *ga20ox1* transcription by binding to the promoter region. Results from the transcription assay showed that either StBEL-05 or POTH1 alone could repress reporter gene activity by more than 50%. The fact that neither POTH1 nor StBEL-05 affected CaMV 35S promoter activity (Figure 24C) confirmed that such repression was not due to inhibition of the general transcription machinery. Direct repression of *GA20 oxidase* gene transcription by the KNOX protein NTH15 has  
30 also been reported in tobacco (Sakamoto et al., "KNOX Homeodomain Protein Directly Suppresses the Expression of a Gibberellin Biosynthetic Gene in the

Tobacco Shoot Apical Meristem,” Genes & Dev. 15:581-590 (2001), which is hereby incorporated by reference in its entirety).

- [0202] Although either StBEL-05 or POTH1 could repress *ga20ox1* promoter in the transcription assay, the KNOX-BEL heterodimers were possibly still formed with endogenous partners to function in tobacco protoplasts. There are three lines of evidence to support this possibility. First, of the seven BEL proteins identified in potato, all seven interacted with four tobacco KNOX proteins (see above). Second, the protein binding domains of the tobacco KNOX NTHs were most important in determining the severity of transgenic plant phenotypes (Sakamoto et al., “The Conserved KNOX Domain Mediates Specificity of Tobacco KNOTTED-1 type Homeodomain Proteins. Plant Cell 11:1419-1431 (1999), which is hereby incorporated by reference in its entirety), implying that interaction with protein partners, most probably the BEL1-like proteins, is essential for KNOX function. Third, the identification of BEL-KNOX binding sites (Smith et al., “Selective Interaction Of Plant Homeodomain Proteins Mediates High DNA-Binding Affinity. Proc. Natl. Acad. Sci. 99:9579-9584 (2002), which is hereby incorporated by reference in its entirety) and the StBEL-05-POTH1 binding site in this study, further implies that the BEL-KNOX dimer is involved in the regulation of target genes. In the transcription assays, constructs of the dominant negatives, StBEL5 $\Delta$ C295 or POTH1 $\Delta$ C122, abolished the repression activity of StBEL-05 or POTH1, respectively (Figure 25). Therefore, StBEL-05 or POTH1 protein alone is not sufficient for the repression of *ga20ox1* promoter. The BEL-KNOX heterodimeric complex is required for repression of transcription to occur.
- [0203] The results above showed that the mutated P1 binding site of the *ga20ox1* promoter did not respond to StBEL-05-POTH1-mediated repression, indicating that this binding site functions as a *cis*-element for the StBEL-05-POTH1 heterodimer. Based on the results from gel-retardation analysis of serial mutations in this site, the mutated promoter was capable of binding with StBEL-05 or POTH1 separately, but not the StBEL-05-POTH1 heterodimer. This is further evidence that it is the BEL-KNOX heterodimer and not the individual BEL or KNOX proteins that affect repression. The interaction of StBEL-05/POTH1 to

affect transcription is summarized in the model of Figure 27. The partner proteins interact through conserved protein binding domains. For StBEL-05, this includes the two amino-terminal helices of the BELL domain and the sky box (Chen et al., “Interacting Transcription Factors From the TALE Superclass Regulate Tuber Formation,” Plant Physiol. (in press) (2003), which is hereby incorporated by reference in its entirety). For POTH1, this includes the KNOX domain with Knox II playing the most significant role (Sakamoto et al., “The Conserved KNOX Domain Mediates Specificity of Tobacco KNOTTED-1 type Homeodomain Proteins. Plant Cell 11:1419-1431 (1999), which is hereby incorporated by reference in its entirety). The sky box contributes to the tandem formation and interacts weakly with Knox I. Interaction between the respective protein binding domains and the spatial arrangement of the first two helices of the homeodomain bring the third helices of both TFs together in a major groove of the DNA helix. Specificity is then provided within the spatial constraints of the three components (StBEL-05, POTH1, and the helical groove) through recognition of the binding motif. In this case, the BEL/KNOX complex may repress transcription by interfering with the binding of critical components of the transcriptional machinery. Other BEL/KNOX complexes may affect gene expression differentially by recognizing other *cis*-elements as a result of slight modifications in protein structure.

[0204] The results indicate that similar to HDs in animals, collaboration of HD proteins to modulate the expression of target genes also occurs in plants. The interaction of HD proteins not only enhances their DNA-binding affinity, but also imparts another level of regulation to these complexes in fine-tuning developmental processes. It is very likely that the numerous potential BEL/KNOX protein interactions participate in a comprehensive system of regulation that coordinates plant growth.

[0205] Although preferred embodiments have been depicted and described in detail herein, it will be apparent to those skilled in the relevant art that various modifications, additions, substitutions, and the like can be made without departing from the spirit of the invention and these are therefore considered to be within the scope of the invention as defined in the claims which follow.